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FIELD AND LABORATORY STUDIES OF DIAGENETIC REACTIONS AFFECTING LIPID RESIDUES ABSORBED IN UNGLAZED ARCHAEOLOGICAL POTTERY VESSELS

by

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ABSTRACT

The effects of decay on lipid residues absorbed in unglazed ceramic potsherds were investigated by: (i) comparing residues recovered from archaeological ceramics and their embedding sediments, and (ii) submitting commodities believed to have been important in antiquity, and presenting contrasting chemical compositions, namely olive oil, cow milk, cow and goat butter, and cod liver oil, to experimental decay under both field and laboratory conditions chosen to promote a range of chemical and biological degradation reactions. The effects of decay on the different commodities investigated were found to be similar and depended mainly on the incubation conditions. Under "hydrolytic" conditions (low to moderate temperatures, moderate moisture), ester hydrolysis was the main degradation reaction, and was accompanied by an overall decrease in the total lipid content of the potsherds. "Oxidative" conditions (high temperature, low moisture) promoted oxidation of the unsaturated components of the residues to form a range of oxidation products, including mono- and dihydroxy fatty acids, α,ω-dicarboxylic acids, short-chain fatty acids, and polar triacylglycerols whose structures and distributions reflected the structures of the unsaturated components they were produced from. Overall, decay affected more especially the more labile components of the residues, namely the low molecular weight and the unsaturated (especially those in the *cis* configuration) fatty acids and triacylglycerols. However, several chemical criteria were found to be useful in the identification of degraded archaeological residues. Firstly, the $\delta^{13}C$ values of individual fatty acids were found to be robust, even in highly degraded residues. The position and configuration of double-bonds in unsaturated fatty acids was found to be affected by decay, albeit in a predictable way involving the preferential loss of the *cis*-configured isomers, so that the resulting distribution still contained information regarding the original composition of the residue. Finally, the recovery and characterisation of oxidation products was shown to provide information regarding the presence and structure of unsaturated acyl lipids in the original aborbed residues, and emphasised the importance of the role played by oxidation during the decay of lipids in archaeological ceramics.

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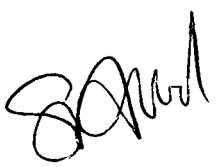
Finally, my thanks go to my father and to all my relatives and friends whose support was invaluable during my time in Bristol.

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree.

Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol.

The dissertation has not been presented to any other University for examination either in the United Kingdom or overseas.

Signed:  Dated: 15 FEB 2002

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List of Abbreviations

CEC:	Cation exchange capacity
DAG:	Diacylglycerol
DMDS	Dimethyl disulphide
FAME:	Fatty acid methyl ester
FFA:	Free fatty acid
GC:	Gas chromatography
GC-C-IRMS:	Gas chromatography-Combustion-Isotope ratio mass spectrometry
GC-MS	Gas chromatography-mass spectrometry
TLE:	Total lipid extract
HTGC:	High temperature gas chromatography
MAG:	Monoacylglycerol
TAG:	Triacylglycerol
TMS:	Trimethylsilylated
UCM:	Unresolved complex mixture

CHAPTER 1: INTRODUCTION

1.1 Lipids

1.1.1 Definitions and structures

Lipids are organic molecules insoluble in water, but soluble in non-polar organic solvents such as chloroform, hexane or methanol (Gurr and Harwood, 1991). This definition embraces a wide range of organic molecules having otherwise no common feature. Lipids are essential components of all living organisms, within which they fulfil a wide range of structural and metabolic roles (Voet and Voet, 1995).

1.1.1.1 Triacylglycerols

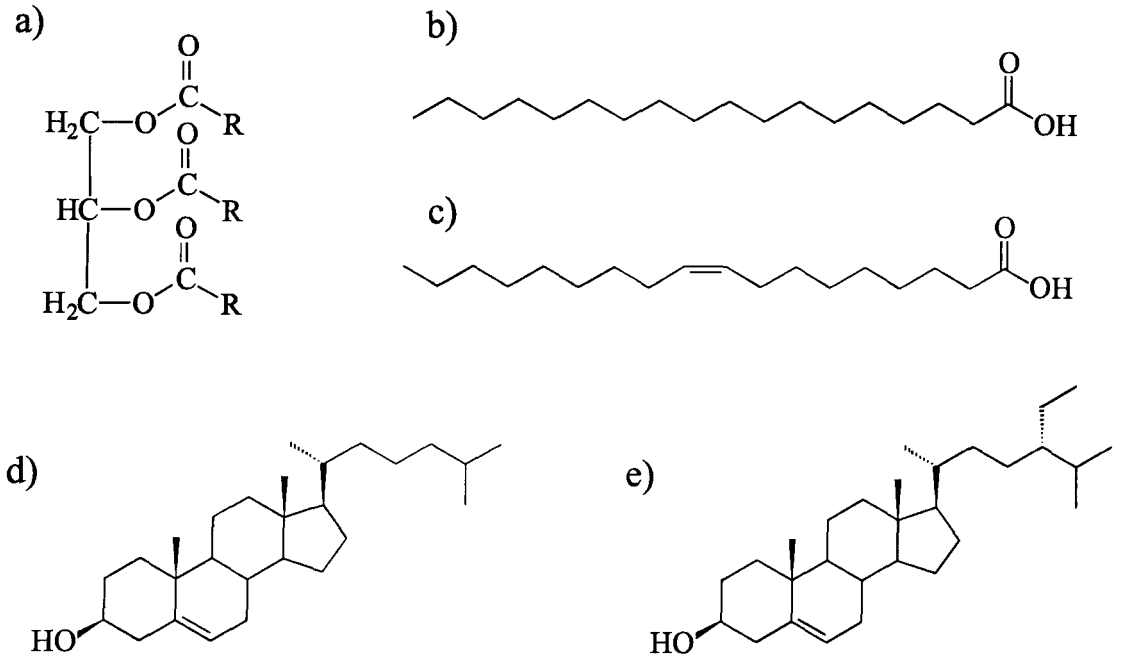


Figure 1.1: Structure of common lipids. a) tristearin (R = $C_{17}H_{35}$), b) stearic acid, c) oleic acid, e) cholesterol and f) β-sitosterol.

The main constituents of oils and fats are triacylglycerols which are esters of three fatty acids with glycerol. Conversely, monoacylglycerols and diacylglycerols are esters of one and two fatty acids respectively, with glycerol. Tristearin or 1,2,3-trioctadecanoylglycerol, is shown in Figure 1a. Triacylglycerols are present in biological systems almost exclusively as energy stores.

1.1.1.2 Fatty acids

Fatty acids, or *n*-alkanoic acids, are long chain carboxylic acids. Most fatty acids contain an even number of acyl carbons because they are synthesised in biological systems from units containing 2 carbon atoms (Voet and Voet, 1995). Fatty acids are usually described by $C_{m:n}$, where m is the length of the carbon chain and n is the number of double-bonds, palmitic acid thereby being $C_{16:0}$. Table 1 gives the trivial names and symbols for common fatty acids encountered herein. The position of the double bonds within the carbon chain is given by Δ^x, which indicates that a double bond is present between the carbon atoms x and x+1 (counting from the carboxyl end of the chain). Unsaturated fatty acids in most animal fats and vegetable oils are present in a *cis*-configuration (Voet and Voet, 1995). Figures 1b and 1c show the structures of two common fatty acids containing 18 acyl carbon atoms, stearic and oleic acids.

1.1.1.3 Sterols

Sterols are alcohols with a complex carbon skeleton containing four fused rings, as shown in Figures 1d and 1e. As in glycerol, the alcohol group of sterols is usually esterified to a fatty acid. The ring system of sterols may be unsaturated and various side chains and groups may be present. Sterols are important structural constituents of cell membranes, and are precursors of steroid hormones and bile acids (Voet and Voet, 1995).

1.1.2 Lipid occurrence in the diet

The ubiquity of lipids in all living organisms causes them to appear in the human diet, either as visible fat (butter, vegetable oils) or hidden fat (fat incorporated in the structure of the food, as in dairy products, nuts, cereal grains; Davídek *et al.*, 1990). Most of man's energy reserves consist of triacylglycerols (Coultate, 1996). It is necessary for man to consume certain fatty acids in his diet, as they can not be synthesised in the body. These essential fatty acids (EFA) include polyunsaturated fatty acids of the n-3 and n-6 classes (with the first double-bond at the carbons 3 and 6, respectively, counting from the methyl end) which are necessary within membranes structures and as precursors of long-chain

Table 1.1: Symbol, systematic and trivial name of common fatty acids.

Symbol	Systematic Name	Trivial Name
	Saturated fatty acids	
$C_{10:0}$	Decanoic	Capric
$C_{12:0}$	Dodecanoic	Lauric
$C_{14:0}$	Tetradecanoic	Myristic
$C_{16:0}$	Hexadecanoic	Palmitic
$C_{17:0}$	Heptadecanoic	Margaric
$C_{18:0}$	Octadecanoic	Stearic
$C_{20:0}$	Eicosanoic	Arachidic
$C_{22:0}$	Docosanoic	Behenic
$C_{24:0}$	Tetracosanoic	Lignoceric
	Monounsaturated fatty acids	
$C_{16:1}\ \Delta^{9}$	*cis*-9-hexadecenoic	Palmitoleic
$C_{18:1}\ \Delta^{9}$	*cis*-9-octadecenoic	Oleic
$C_{18:1}\ \Delta^{11}$	*cis*-11-octadecenoic	Vaccenic
$C_{20:1}\ \Delta^{9}$	*cis*-9-eicosenoic	Gladoleic
$C_{22:1}\ \Delta^{13}$	*cis*-13-docosenoic	Erudic
$C_{24:1}\ \Delta^{15}$	*cis*-15-tetracosenoic	Nervonic
	Polyunsaturated fatty acids	
$C_{18:2}\ \Delta^{9,12}$	All *cis*-9, 12 –octadecadienoic	Linoleic
$C_{18:3}\ \Delta^{9,12,15}$	All *cis*-9, 12 ,15 -octadecatrienoic	α-Linolenic
$C_{18:3}\ \Delta^{6,9,12}$	All *cis*-6, 9, 12-octadecatrienoic	γ-Linolenic

polyunsaturated fatty acids and of some hormones, such as prostaglandins (Coultate, 1996; Spector, 1999). Lipids also include the fat soluble vitamins A, D, E and K. Finally, lipids contribute to the palatability of food (i.e. texture, odour and taste) and might therefore be deliberately added to food commodities during cooking (Coultate, 1996).

1.2 Incorporation of lipids into unglazed archaeological ceramics

During the processing of animal and vegetable commodities in unglazed ceramic vessels, the action of heat and friction (caused by stirring), above all in the presence of water, causes the disruption of cell walls and the subsequent release of the lipids, which may then drip to the bottom of the vessel or form a suspension in water and deposit along the

vessel walls. Lipids are then absorbed in the ceramic matrix where they undergo little thermal damage (Röttlander, 1990), except in the base of the vessel, where heat is usually directly applied. Absorption of lipids from food commodities processed inside a vessel creates a concentration gradient across the vessel wall that has been observed in an amphora used to store olive oil (Condamin *et al.*, 1976; Condamin and Formanti, 1978) and in a potsherd originating from a vessel used to store or process a vegetable dye (Biek, 1963). Furthermore, previous experimental work showed that the lipid accumulation pattern along the vessel profile was characteristic of the way the commodities had been processed (Charters, 1996; Charters *et al.*, 1993 and 1995; Evershed *et al.*, 1995). Upon boiling, lipids are mobilised in the aqueous phase, float to the surface and, thus, are preferentially deposited in the highest part of the vessel, whereas roasting causes the lipids to drip to the bottom of the vessel, where they are preferentially absorbed (Charters, 1996; Charters *et al.*, 1993 and 1995; Evershed *et al.*, 1995). When an excessive amount of heat is applied, above all in the absence of water, food commodities may burn and form encrusted charred residues on the inside walls of the vessels (Rottländer and Schlichtherle, 1979; Hastorf and deNiro, 1985; Oudemans and Boon, 1991; Sherriff *et al.*, 1995).

Lipid residues can also be absorbed in unglazed ceramics through the exploitation of oils and fats for non-culinary purposes. For example, beeswax, vegetable oils or animal tallow have been widely used as fuel in clay lamps (Evershed *et al.*, 1997a; Bland, 1999; Mottram *et al.*, 1999), and other substances containing lipids, such as resins and tars, have been applied intentionally to the walls of porous vessels to seal them and allow them to be used for the storage or processing of liquids (Beck *et al.*, 1989; Cheape, 1988).

1.3 Diagenetic processes affecting lipid residues during vessel use and burial

Once buried, the porous matrix of unglazed potsherds offers a very favourable environment for lipid preservation. Absorption limits the access of microorganisms to the residues and alters the availability of the latter as bacterial substrates (Evershed, 1993). Furthermore, conditions within the porous ceramic matrix are different from that of the surrounding soil, as, for instance, levels of organic and inorganic nutrients, pH, water and

oxygen flows are altered (Alexander, 1999). Finally, absorption limits loss by diffusion into soil water, and, thereby, enhances preservation. However, lipids contain a variety of functional groups, especially ester groups and double-bonds, which make them susceptible to degradative reactions during vessel use and burial.

Previous investigators have demonstrated that it was possible to submit lipids absorbed in unglazed potsherds to experimental burial under laboratory conditions, and that the resulting residues resembled those observed in archaeological ceramics (Charters, 1996; Charters *et al.*, 1995; Dudd *et al.*, 1998; Malainey *et al.*, 1999b and 1999c). Comparisons between fresh oils and fats and degraded residues recovered either from experimental or archaeological potsherds have enabled some insights to be gained into the various degradation reactions affecting absorbed lipid residues during vessel use and burial.

1.3.1 Ester hydrolysis

Triacylglycerol hydrolysis (Figure 1.2) mainly yields free fatty acids; mono- and diacylglycerols are formed only in minor quantities because partial acylglycerol hydrolysis is very fast (Charters, 1996; Dudd *et al.*, 1998, Dudd, 1999; Davídek *et al.*, 1990). Free fatty acids contribute to the rancidification of food, especially in dairy products (Choi and Jeon, 1993; Stead, 1986). During food storage, hydrolysis is usually driven by lipolytic enzymes produced by contaminating microorganisms. Lipolytic activity is catalysed by metal ions, such as iron, magnesium and manganese. Microbial lipases are also involved in triacylglycerol transesterification, during which acyl moieties are exchanged between positions on the glycerol backbone (Davìdek *et al.*, 1990; Bugg, 1997).

During vessel burial, triacylglycerol hydrolysis has been reported to be responsible for most of the alteration of the composition of the absorbed residues (Charters, 1996; Charters *et al.*, 1995; Dudd *et al.*, 1998; Dudd, 1999). The incorporation of microbial lipids (ergosterol and branched fatty acids $C_{15:0}$ and $C_{17:0}$) during the laboratory degradation of milk, olive oil and pure tristearin (Dudd *et al.*, 1998; Dudd, 1999) indicated that hydrolysis was enzymatically driven, at least initially, when the lipid concentration on the surface of the potsherd was high. During these latter experiments, the total lipid content of the potsherds decreased with incubation time as lipids were consumed by microorganisms (Charters, 1996; Charters *et al.*, 1995; Dudd *et al.*, 1998;

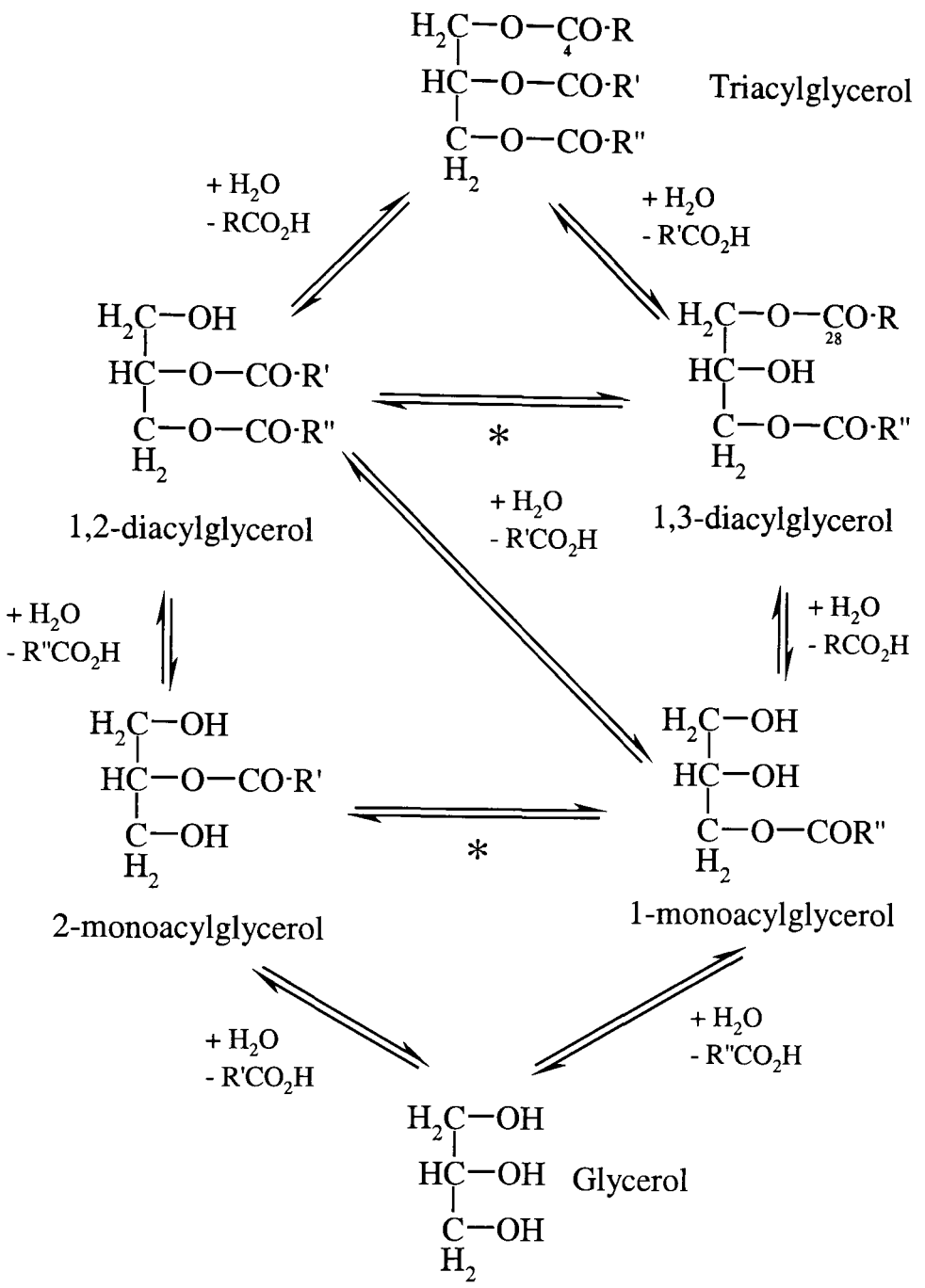


Figure 1.2: Triacylglycerol hydrolysis (Davídek *et al.*, 1990). Transesterification reactions are indicated by *.

Dudd, 1999). Charters and co-workers (Charters, 1996; Charters *et al.*, 1995) observed the more rapid decay of animal-derived triacylglycerols compared to that of vegetable waxes during experimental decay of a residue obtained by sequential boiling of cabbage leaves and lamb meat in a ceramic vessel. Preferential hydrolysis of short-chain acyl moieties (<12 acyl carbon atoms) was observed during the experimental decay of milk fat (Dudd *et al.*, 1998, Dudd, 1999), thus causing degraded dairy fats to resemble degraded adipose fats and making their identification difficult. Triacylglycerol hydrolysis was observed in several ethnographic vessels with well-documented use history (Charters, 1996; Dudd, 1999), indicating that this reaction can also take place within the ceramic walls during vessel use.

1.3.2 Ketonic decarboxylation

Ketonic decarboxylation of fatty acids, a kind of head to head condensation reaction, has been demonstrated to be responsible for the formation of a homologous series of long-chain ketones dominated by C_{30}, C_{33} and C_{35} in the ratio 1:2:1 from fatty acids absorbed in unglazed ceramic (Figure 1.3, Evershed *et al.*, 1995b; Raven *et al.*, 1997). Such ketones are commonly encountered in archaeological residues identified as degraded animal fats, and had previously been erroneously identified as plants ketones.

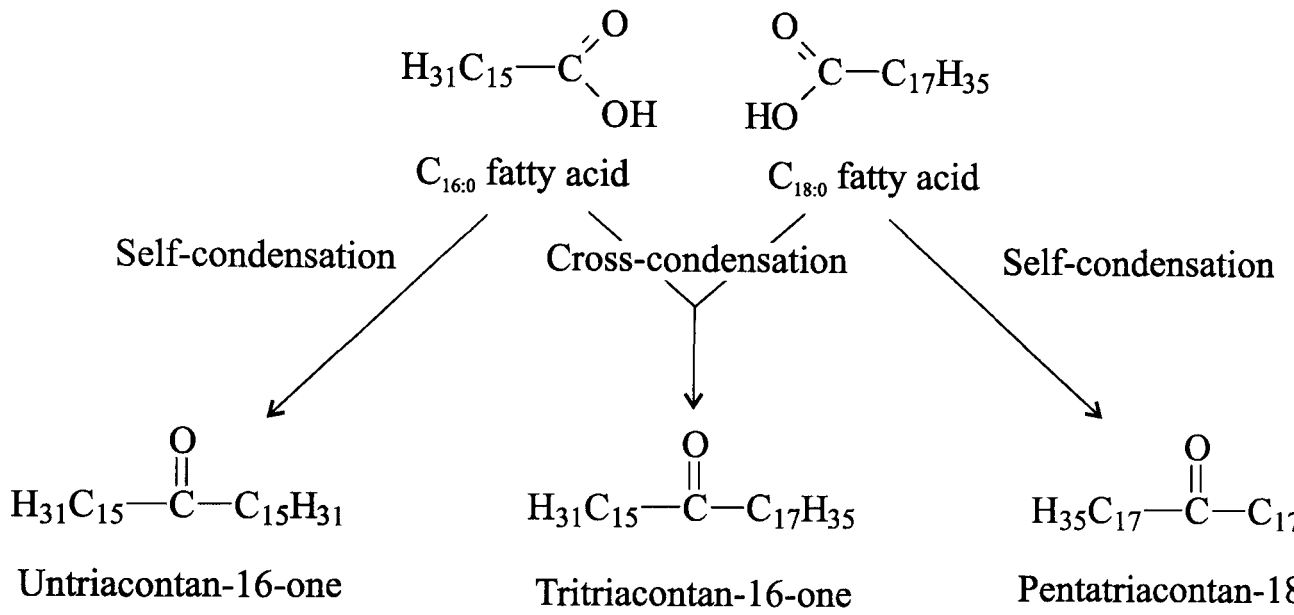


Figure 1.3: Fatty acid ketonic decarboxylation (Evershed *et al.*, 1995b; Raven *et al.*, 1997).

1.3.3 Cracking

Shimoyama and co-workers (1993) observed the formation of a series of short-chain fatty acids dominated by nonanoic acid ($C_{9:0}$) and of γ-lactones by reacting oleic acid with oxygen in the presence of clay and water. They observed similar compounds in residues recovered from archaeological ceramics and identified them as deriving from the processing of olive oil at high temperature (Shimayama *et al.*, 1995). The cracking of fatty acids to yield short chain fatty acids is believed to occur at very high temperature during the frying or roasting of food, above all in the presence of metal catalysts (Davídek *et al.*, 1990)

1.3.4 Unsaturated lipids oxidation

1.3.4.1 Radical oxidation

Radical oxidation of unsaturated lipids proceeds in three steps, namely initiation, propagation and termination (Figure 1.4). Initiation proceeds by abstraction of an allylic (α to a double-bond) hydrogen from an unsaturated fatty acid or sterol (I; Hamilton *et al.*, 1997), catalysed by the action of heat or by metal ions, free radicals or peroxides. During the propagation step, the free radical (II) thereby formed reacts with atmospheric oxygen O_2 to form a peroxy radical (III) which reacts with intact lipids (IV) to form a hydroperoxide (V) and a lipid radical (VI), which can, in turn, react with more O_2. Termination takes place when radical species either react together to form a non-radical compound, or react with a compound to form either a non-radical product or a stable radical. Such compounds are called antioxidants (Coultate, 1996).

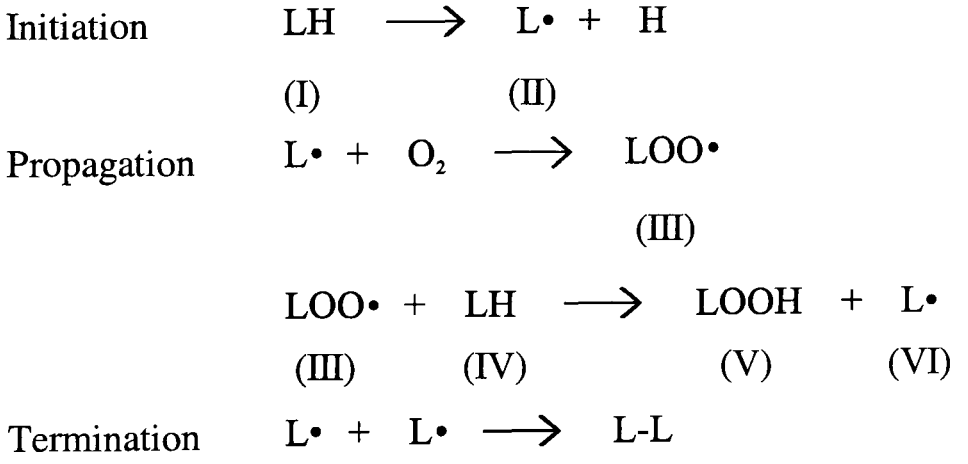


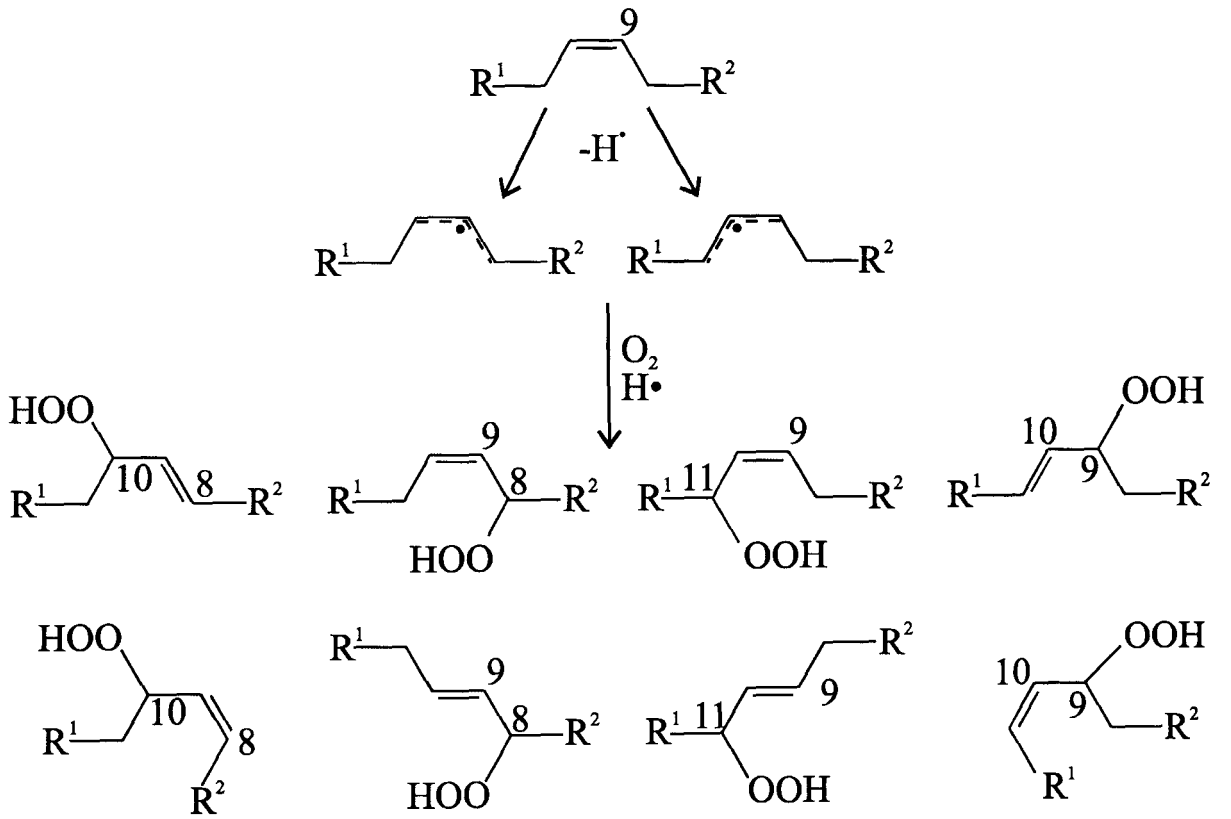
Figure 1.4: Lipid radical oxidation (Hamilton *et al.*, 1997). Compound identities are explained in the text.

1.3.4.2 Photosensitized oxidation

Oxygen can become excited to the singlet state ($^1O_2$) by transfer of energy from a sensitizer such as chlorophyll or riboflavin (very important in milk), in the presence of light, and react with lipids to form hydroperoxides (Coultate, 1996). Hydroperoxides formed by photosensitized oxidation differs from those formed by radical oxidation by the position of the hydroperoxide functions and of the double-bonds and in the proportion of the various isomers, as illustrated by the example of oleic acid (Figure 1.5; Frankel,

1998). Photosensitized formation of hydroperoxides is thought to play an important role in the initiation step of radical lipid oxidation.

a)



b)

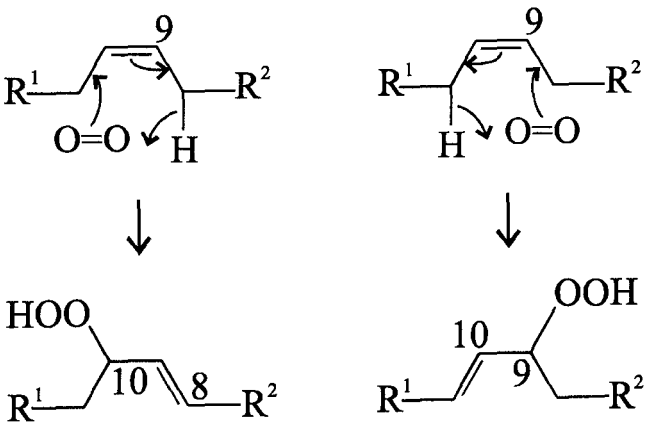


Figure 1.5: a) Oleic acid radical oxidation, b) oleic acid photosensitised oxidation (Frankel, 1998). $R^1 = C_7H_{15}$, $R^2 = C_7H_{13}O_2$.

1.3.4.3 Action of lipoxygenases

Lipoxygenases are enzymes common in plant and animal tissues and in microorganisms (Davídek *et al.*, 1990; Hamilton *et al.*, 1997). They catalyse the formation of hydroperoxides with positional and geometrical specificity (Mlakar and Spiteller, 1996a; German *et al.*, 1992). The hydroperoxides can then decompose to form aldehydes that

contribute to the flavour of food, but also participate in the initiation step of radical oxidation of unsaturated lipids (Flick *et al.*, 1992). These reactions are accompanied by shifts in the double-bond positions to form conjugated polyunsaturated species (Davídek *et al.*, 1990).

1.3.4.4 Hydroperoxide decomposition

Hydroperoxides are very reactive species and undergo decomposition and rearrangement to form a wide range of volatile and non-volatile products. Hydroperoxide decomposition is catalysed by the presence of metals, such as iron, copper, manganese and cobalt. Scission of the carbon-carbon bond α to the hydroperoxide function leads to the formation of short chain alkanes, alkenes and aldehydes. Scission of the oxygen-oxygen bond within the hydroperoxide followed by molecule rearrangement leads to the formation of ketones, epoxides and epidioxides (Coultate, 1996; Gunstone *et al.*, 1986; Frankel, 1998 and references therein). Finally, polymers are formed by formation of peroxy or peroxidic linkages between fatty acids or triacylglycerols, or *via* Diels-Alder reactions to form cyclic compounds, as observed in deep-frying oils and in drying oils used in paints (Frankel, 1998 and references therein; Martin *et al.*, 1998; Hopia, 1993a and 1993b; Muizebelt and Nielen, 1996; Mills and White, 1994). Polymerisation of lipid residues associated with ceramic potsherds is thought to create a highly polymerised network from which lipids are not recovered by traditional solvent-extraction (Evershed *et al.*, 1992). Regert and co-workers (1998) were able to release oxidation products (i.e. a range of α,ω-dicarboxylic acids, dominated by the C_9 compound, and 9,10-dihydroxyoctadecanoic acid) believed to be part of this polymerised network by submitting solvent-extracted potsherds to saponification. The release of "bound" oxidation products by saponification of the extracted ceramic residue was fully exploited by Bland (1999) in the identification of vegetable oils used as fuel in clay lamps. In both cases, the characterisation of oxidation products in both the solvent-extractable and "bound" fractions provided valuable information regarding the original presence and structure of unsaturated lipids in the intact residue.

1.3.5 β-oxidation

β-oxidation is the stepwise degradation of fatty acids in living organisms (Figure 1.6). Fatty acids are first activated by reaction with coenzyme-A. A double bond with the *trans*-configuration is then formed in position 2. This double bond is then hydrated to form an alcohol in position 3 which is then turned into a ketone. Scission of the bond between the carbons 2 and 3 yields acetyl-CoA which is used in energy production and a fatty acid containing two less carbon atoms then the original one which can then react with coenzyme-A and start a new cycle. (Voet and Voet, 1995; Gurr and Harwood, 1991)

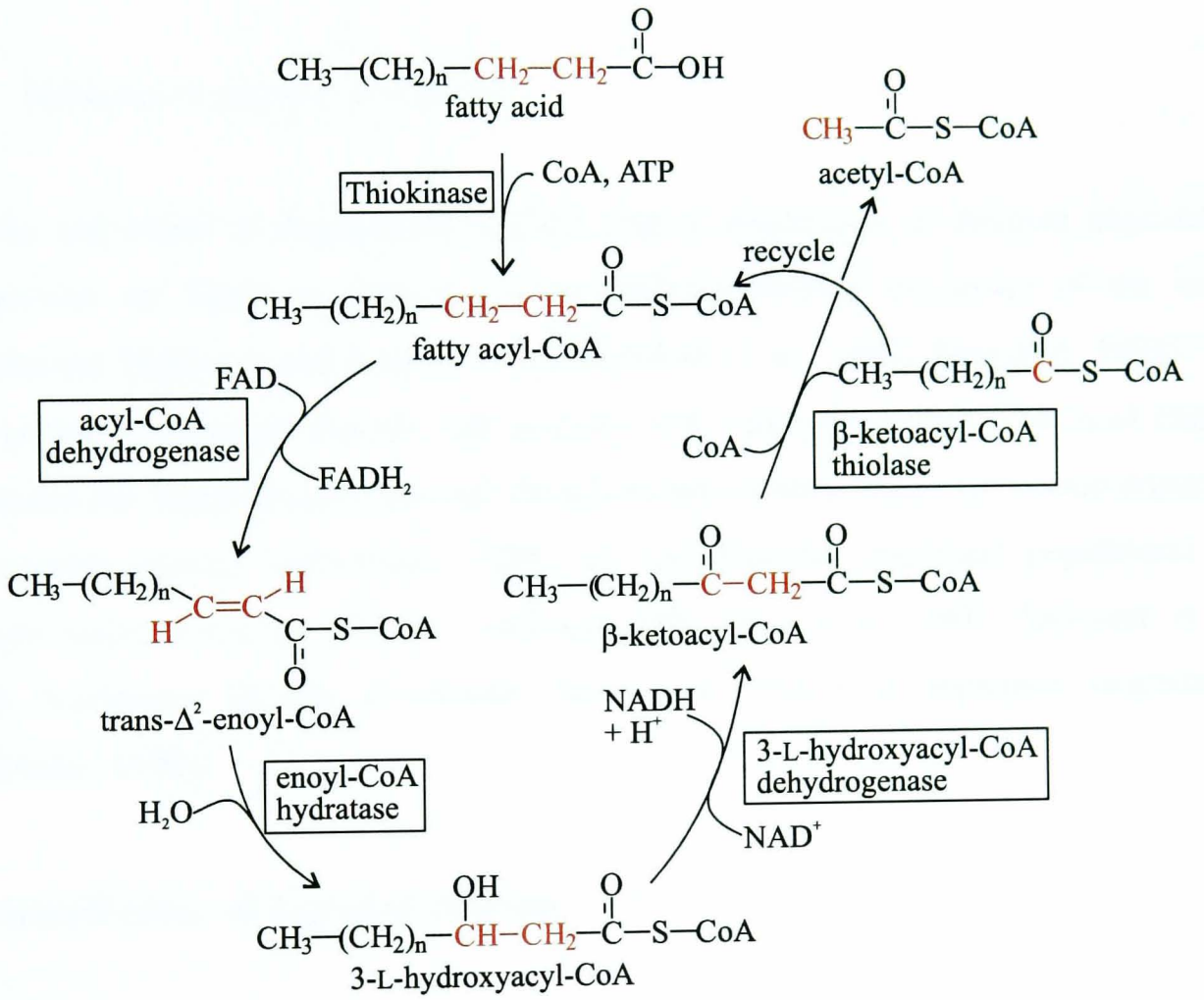


Figure 1.6: Mechanism of lipid β-oxidation (Voet and Voet, 1995).

1.3.6 Adipocere formation

Adipocere formation is observed in human remains buried under waterlogged and acidic conditions (Evershed *et al.*, 1992; Evershed, 1990; Mayer *et al.*, 1997), and in bog butters (Thornton *et al.*, 1970). Adipocere is thought to result from microbial degradation of adipose fat and consists mainly of saturated free fatty acids with even-numbered carbon

atoms ranging from $C_{14:0}$ to $C_{18:0}$, dominated by $C_{16:0}$. Adipocere also usually contains significant amounts of 10-hydroxyoctadecanoic acid, which is thought to be produced by hydration of oleic acid across the double-bond (Mayer *et al.*, 1997; Evershed *et al.*, 1992; Evershed, 1990). Experimental adipocere formation showed the depletion of unsaturated fatty acids and the concomitant increase in abundance of the saturated fatty acids containing two less carbon atoms (Den Dooren de Jong, 1961). β-oxidation was thought to be involved in that process but it is unlikely that it would stop after only one cycle (Morgan *et al.*, 1983; Mills and White, 1994). Scrambling of the double-bond position presumed to result from biohydrogenation of the remaining unsaturated fatty acids was reported in human adipocere (Evershed, 1990)

1.3.7 Influence of external conditions

The rate and extent of degradation and the relative importance of different degradation mechanisms are likely to depend on the physico-chemical properties of the burial environment (Eglinton and Logan, 1991; Evershed *et al.*, 1992; Evershed, 1993). The local geology influences the pH, soil aeration and water movement. The local climate determines the burial temperature and the soil water content. These factors are important for bacterial activity (Alexander, 1999), as, for example, microbial populations are different under acidic or alkaline conditions (Moucawi *et al.*, 1981; DeLaune *et al.*, 1981). Variations of the conditions have been shown to stimulate degradation (Rottländer, 1990).

1.4 Identification of degraded residues

The identification of degraded residues recovered from archaeological potsherds has traditionally been achieved after chemical degradation (saponification) of the residues to yield free fatty acids that were quantified by gas chromatography or high performance liquid chromatography and whose relative proportions were compared with that found in fresh oils and fats. Previous investigators were thereby able to recognize degraded vegetable oils (Condamin and Formenti, 1978; Condamin *et al.*, 1976; Passi *et al.*, 1981; Shimoyama *et al.*, 1995), and animal fats (Bourgeois and Gouin, 1995; Rottländer and

Schlichtherle, 1979). Pyrolysis gas chromatography mass spectrometry (Oudemans and Boon, 1991) of charred food remains enabled the identification of lipids, sugars and proteins markers and of a regular pattern of *n*-alkanes and *n*-alkenes believed to be an aliphatic network formed during food processing at high temperature. Infra red (Beck *et al.*, 1989, Condamin and Formenti, 1978) and nuclear magnetic resonance (Sherriff *et al.*, 1995) spectroscopy were applied to degraded residues to identify functional groups. Recently, high temperature gas chromatography and high temperature gas chromatography-mass spectrometry have allowed the characterisation of a wide range of components of degraded residues without prior fractionation or chemical degradation (Evershed *et al.*, 1990). The subsequent identification of components specific to certain oils and fats, such as sterols (Evershed *et al.*, 1992; Heron *et al.*, 1991; Heron and Evershed, 1993) and wax esters (Evershed, 1993; Evershed *et al.*, 1997a) has proved to be a useful tool in the identifications of degraded residues. More recently, determination of the stable carbon isotope ratios of bulk residues was used to distinguish between encrustations deriving from C3 and C4 plants and carnivores and omnivore fats (Hastorf and deNiro, 1985, Sherriff *et al.*, 1995). Compound specific stable isotope studies ($C_{18:0}$ and $C_{16:0}$) has enabled distinctions to be made between dairy and adipose fats, and between ruminant and non-ruminant adipose fats (Mottram *et al.*, 1999; Evershed *et al.*,1994 and 1997b; Dudd and Evershed, 1998; Dudd, 1999; Dudd *et al.*, 1999). Determination of the double-bond position and configuration in unsaturated fatty acids provided further evidence regarding the distinction between ruminant and non-ruminant fats (Mottram *et al.*, 1999; Evershed *et al.*, 1997b; Dudd, 1999).

1.5 Aims of the thesis

The identification of degraded lipid residues recovered from archaeological ceramics is based on comparing a range of distributional and molecular properties between degraded residues and fresh fats and oils. As degradation processes affect lipids with different structures to different extents, it is fundamental to monitor the composition of lipid residues during decay in order to bring more confidence to archaeological interpretations.

Furthermore, the effect of decay on absorbed lipids is very likely to be vary depending on the burial conditions. A better understanding of the influence of the burial conditions on decay would enhance our understanding of the mechanisms of decay/preservation in relation to the recovery of organic residues from different sites and contexts within sites. The main aim of this thesis is therefore to submit lipids (representative of commodities believed to have been commonplace in Antiquity) absorbed in unglazed ceramics to experimental decay under both laboratory and field conditions and, thereby:

(i) Identify the main processes affecting lipids absorbed in unglazed ceramics.

(ii) Monitor the changes of the main chemical properties used in the identification of archaeological residues during decay (e.g. triacylglycerol and free fatty acid distributions, stable carbon isotope ratios of individual fatty acids and double-bond position and configuration in unsaturated fatty acids).

(iii) Monitor the fate of unsaturated lipids, found in abundance in plant and fish oils, and short-chain fatty acids, characteristic of dairy fats, during decay.

(iv) Develop new diagnostic criteria based on degradation products (e.g. oxidation products) for the identification of lipids associated with unglazed ceramics.

(v) Investigate the influence of the burial conditions on the rate and extent of decay.

CHAPTER 2: EXPERIMENTAL PROCEDURES

2.1 Samples

2.1.1 Archaeological Samples

Figure 2.1: Map of mainland Great Britain showing the location of the archaeological and experimental sites mentioned in this thesis.

2.1.1.1 Eton

Unglazed potsherds were recovered from an archaeological excavation in Eton, Berkshire, UK (site LERW96, Figure 2.1). Investigation focused on a Saxon pit (Pit 2102), which yielded 8 potsherds. Pit 2102 was circular (1.20 m in diameter) and 86 cm deep. Contrasts between the filling sediments defined 2 contexts: context 2102 (bottom 40 cm) and context 2100 (top 40 cm). An Iron Age pit (pit 668) was briefly excavated and yielded 3 potsherds. Each potsherd was numbered in order of recovery and placed in a plastic bag, alongside the embedding sediments. One potsherd (2102/3), recovered from the Saxon pit, was in an advanced stage of degradation and was collected embedded in the burial sediments.

2.1.1.2 Crick

Potsherds were recovered from the archaeological excavation of an Iron Age site at Covert Farm (DIRFT East), Crick, Northamptonshire, UK (Figure 2.1). This site is an extensive settlement consisting of penannular ring ditches and gullies and post-hole settings. Investigation focused on the extremities of the ditches and gullies, which yielded 17 potsherds which were sampled as described above.

2.1.2 Experimental potsherds

A wheel-thrown replica vessel made from a mixture of Pot Clay (1137; Keuper Marl, Staffordshire) and sand (3:1, v/v) was broken into potsherds using a chisel and hammer. Potsherds for the field decay experiments were drilled through using a 4 mm masonry drill bit.

2.2 Experimental potsherd dosage

2.2.1 Modern oils and fats used for potsherd dosage

Commercial extra virgin olive oil, pure cod liver oil, organic full fat cow milk and organic cow butter were used for potsherd dosage. Cow milk was either used directly or after heat treatment by boiling it down to 1/3 of its original volume. Goat butter was prepared in the laboratory from commercial goat cream by churning in a closed jar with a small amount of water.

2.2.2 Potsherd dosage

2.2.2.1 Olive oil

Potsherds (*ca.* 2 g) were dosed by sonication in olive oil solutions (25, 50, 75, 100, 250, 500 mg ml^{-1} of commercial extra virgin olive oil in dichloromethane, and pure olive oil) for various periods of time (up to 120 min) to investigate the effect of solution concentration and sonication time on the amount of lipids absorbed by the potsherd. Potsherds (*ca.* 4 g) destined at experimental decay were dosed in an olive oil solution

(100 mg ml^{-1} in dichloromethane) for 2 x 15 min. One potsherd was kept for analysis at Day 0 and the others were submitted to experimental decay as described below.

2.2.2.2 Dairy fats

Potsherds (*ca.* 2 or 4g) were dosed in boiling milk or butter for various periods of time (15 and 60 min) after which they were removed and dried with paper tissues, before being submitted to lipid extraction to establish the optimum time for lipid absorption. Potsherds destined at experimental decay were dosed for 30 min.

2.2.2.3 Cod liver oil

Potsherds (*ca.* 4g) were dosed in cod liver oil solution (100 mg g^{-1} in dichloromethane) for 2 x 15 min.

2.3 Experimental decay experiments

2.3.1 Laboratory experiments

Potsherds and mushroom compost (mushroom humix manure, Magnolia Brand) were added to Schott bottles of various volumes in the proportion of 15 g compost per potsherd. Water was added to the flasks to create different moisture levels. For decay under aerobic conditions, the neck of each bottle were plugged with cotton wool whereas, for anaerobic conditions, it was tightly closed with a rubber cap and a screw top; the bottles were then incubated at constant temperature (incubators or constant temperature rooms). Details of the laboratory experiments and controls are given in the Tables 2.1 and 2.2. Two experiments were set up using blank potsherds in order to investigate the absorption of lipids during burial (Table 2.3).

Table 2.1: Details of the laboratory experiments investigating the decay of olive oil.

Experiment number	Temperature / °C	Water content	Aeration conditions	Sampling intervals / days
1	7	Low[1]	oxic[4]	0, 3, 6, 9, 13, 16, 21, 24, 29, 35, 42, 49, 62, 133, 182, 267, 365, 729
2	7	Medium[2]	oxic[5]	0, 3, 6, 9, 13, 16, 21, 24, 29, 35, 42, 49, 62, 133, 182, 267, 365, 729
3	7	High[3]	oxic	0, 3, 6, 9, 13, 16, 21, 24, 29, 35, 42, 49, 62, 133, 182, 267, 365, 729
4	25	Low	oxic	0, 2, 4, 7, 10, 14, 17, 22, 25, 30, 36, 42, 50, 63, 134, 183, 267, 366, 730
5	25	Medium	oxic	0, 2, 4, 7, 10, 14, 17, 22, 25, 30, 36, 42, 50, 63, 134, 183, 268, 366
6	25	Low	anoxic	0, 2, 4, 7, 10, 14, 17, 22, 25, 30, 36, 42, 50, 63, 134, 183, 266
7	25	High	anoxic	0, 2, 4, 7, 10, 14, 17, 22, 25, 30, 36, 42, 50, 63, 134, 183, 274, 366
8	55	Low	oxic	0, 1, 2, 5, 7, 12, 15, 20, 23, 28, 34, 41, 48, 61,132, 181, 266, 364, 728
9	55	Medium	oxic	0, 1, 2, 5, 7, 12, 15, 20, 23, 28, 34, 41, 48, 61,132, 181, 266, 364
10	55	High	oxic	0, 1, 2, 5, 7, 12, 15, 20, 23, 28, 34, 41, 48, 61,132, 181, 266, 364
11	25	-[6]	oxic	0, 4, 8, 13, 18, 25, 32, 42, 50, 75, 95, 125, 162, 180, 235, 320, 412

[1]Sherds buried in mushroom compost in the proportion of 15 g compost per potsherd. No water was added.
[2]10 ml of water per 15 g of compost was added to the incubation flask.
[3]20 ml of water per 15 g of compost was added to the incubation flask.
[4]Air allowed to diffuse into the flask.
[5]Flask was purged with N_2 and tightly closed.
[6]Sherds were incubated in a clean flask containing no compost.

Table 2.2: Details of the laboratory experiments investigating the decay of dairy fats and of cod liver oil.

Experiment number	Commodity	Temperature / °C	Aeration conditions	Moisture content	Sampling intervals / days
12	Cow butter	30	Oxic[1]	-[2]	0, 15, 35, 50, 75, 142, 200, 300, 349
13	Cow butter	30	Oxic	Medium[3]	0, 15, 35, 50, 75, 142, 200, 300, 349
14	Goat butter	30	Oxic	Medium	0, 15, 35, 50, 75, 142, 200, 300, 348
15	Cod liver oil	30	Oxic	Medium	0, 1,2, 12, 16, 27, 40, 60, 90, 127, 148, 210, 286, 330, 399
16	Cod liver oil	30	Oxic	Low[4]	0, 1, 2, 12, 16, 27, 40, 60, 90, 127, 148, 210, 286, 330, 399
17	Cod liver oil	30	Oxic	-	0, 3, 23, 50, 85, 119, 265

[1]Air is allowed to diffuse into the flask.
[2]Sherds are incubated in a clean bottle containing no compost
[3]10 ml of water per 15 g of compost was added to the incubation flask.
[4]Sherds buried in mushroom compost in the proportion of 15 g compost per potsherd. No water was added.

Table 2.3: Details of the laboratory experiments investigating the absorption of lipids from the burial environment by blank potsherds.

Experiment number	Temperature / °C	Water content	Aeration conditions	Sampling intervals / day
18	25	Low[1]	Oxic[3]	0, 2, 4, 7, 10, 14, 17, 22, 25, 30, 36, 43, 63, 183, 268, 366.730
19	25	High[2]	Oxic	0, 2, 4, 7, 10, 14, 17, 22, 25, 30, 36, 43, 63, 183, 268, 366, 730

[1]Sherds buried in mushroom compost in the proportion of 15 g compost per potsherd. No water was added.
[2]20 ml of water per 15 g of compost was added to the incubation flask.
[3]Air is allowed to diffuse into the flask.

2.3.2 Field experiments

Details of the field experiments investigating the decay of dairy fats are given in Table 2.4. For each experiment, two potsherds were kept for analysis at day 0. Potsherds were prepared by threading garden wire (10 cm) through them and a bright plastic garden tag, colour-coded for each commodity, was attached at the other end of the garden wire. Potsherds were subsequently buried on site in the most microbially active soil horizon, just below the root mat (Figure 2.2), in such a way that the plastic label was above the soil surface and clearly visible (Figure 2.3). Soil samples were taken from each burial location to enable the determination of the burial environment physico-chemical properties.

Table 2.4: Details of the field experiments investigating the decay of dairy fat (Westbury-on-Trym, Bristol, UK; see Figure 2.1 for location)

Experiment number	Commodity decayed	Sampling intervals / days
20	Cow milk	0, 2, 5, 10, 15, 25, 50, 75, 162, 272
21	Cow butter	0, 2, 5, 10, 15, 25, 50, 75, 162, 272,386
22	Pasteurised cow milk	0, 2, 5, 10, 18, 25, 54, 78, 141, 251

Figure 2.2: Experimental plot, showing the potsherds placed below the root mat.

Figure 2.3: Experimental plot after the turf has been put back on top of the potsherds, leaving the tags clearly visible.

2.4 Sample cleaning and extraction

2.4.1 Handling and pre-treatment

2.4.1.1 Potsherds

Disposable rubber gloves were worn during potsherd processing in order to prevent contamination from finger grease. Potsherds were rinsed with tap water and brushed free of adhering soil. They were then allowed to dry in a dessicator, cleaned with an electric modelling drill and stored in the freezer (-20°C), until required for analysis.

2.4.1.2 Burial soils

Soil samples were transferred into glass bottles and freeze-dried. They were then ground in order to break up any aggregates and stones and vegetable fragments of size > 2 mm were removed.

2.4.2 Lipid extraction

2.4.2.1 Solvent-extractable fraction from potsherds

A portion of the ceramic (*ca.* 2 g) was reduced to a powder with a mortar and pestle. It was then transferred to a solvent-washed vial to which a known amount (10 µl to 500 µl) of internal standard (*n*-tetratriacontane in hexane, 1 mg ml^{-1}) was added, followed by the extraction solvent (chloroform/methanol, 2:1 v/v, 10 ml). The vial and its content were sonicated (2 x 15 min) then the extraction solvent decanted to a solvent-washed centrifuge-tube. The centrifuge tubes (samples were generally processed in groups of 6) were then spun (2000 rpm, 20 min) in a bench-top centrifuge (Mistral 1000, MSE). The supernatant from each tube was transferred to a solvent-washed vial and the solvent blown down under a gentle stream of nitrogen to afford the total lipid extract (TLE). The TLE was dissolved in chloroform/methanol (2:1 v/v, 500 µl). A fifth aliquot was filtered through a silica (silica gel 60) column and the filtrate collected in a solvent-washed vial.

An analytical blank, which contained no potsherd material, was processed concurrently with each set of samples to check for the presence of contamination.

2.4.2.2 Solvent-extractable fraction from soil samples

Soil samples (30 g) to which was added a known amount (50-200 µl) of internal standard (*n*-tetratriacontane in hexane, 1 mg ml^{-1}) were Soxhlet-extracted with chloroform/ methanol (2:1 v/v, 200 ml) for 24 h. The solvent was then removed by rotary-evaporation to afford the TLE, of which a fifth aliquot was filtered through silica prior derivatisation and analysis by gas chromatography.

2.4.2.3 Alkaline hydrolysis of the "bound" fraction from potsherds

A portion (*ca.* 1 g) of the extracted powdered potsherd was transferred to a 50 ml round-bottomed flask. 10 ml of 0.5M sodium hydroxide in methanol/water (9:1, v/v) was added and the mixture heated under reflux at 70° C for 90 min with continuous stirring. After cooling, the saponified mixture was centrifuged (2000 rpm, 20 min). An aliquot (3 ml) was acidified with hydrochloric acid (1 M) and extracted with chloroform (3 x 3 ml). The extracts were combined and the solvent evaporated under a stream of nitrogen.

2.5 Extract fractionation and derivatisation

2.5.1 Acid/Neutral separation

An aliquot of the TLE was applied on a solvent-conditioned (methanol/chloroform, 2:1 v/v, 12 ml) aminopropyl column (Bond Elute solid phase extraction cartridge; Varian sample preparation product). The neutral fraction was eluted with dichloromethane/isopropanol (2:1, v/v, 12 ml) and the free fatty acids with 1% v/v acetic acid in diethyl ether (12 ml). Solvents were removed under a gentle stream of nitrogen.

2.5.2 Saponification

An aliquot of the TLE was placed in a derivatisation tube with sodium hydroxide (0.5 M in methanol, 2 ml) and heated (70° C, 1 h). After cooling, the solution was acidified

(hydrochloric acid, 2 M) and the fatty acids extracted with hexane (3 x 2 ml). The extracts were combined and the solvent removed under a gentle stream of nitrogen.

2.5.3 Transmethylation

Free fatty acids were placed in a derivatisation tube with BF_3-methanol (100 µl, 14% solution, BDH) and heated (70° C, 1 h). After cooling, water (2 ml) was added and the fatty acids methyl esters (FAMEs) were extracted with ether (3 x 2 ml). The extracts were combined and the solvent was blown down under a gentle stream of nitrogen. FAMEs were kept in the fridge (4° C) until required for analysis.

2.5.4 Dimethyl disulphide (DMDS) derivatives

DMDS (100 µl) and iodine in diethyl ether (6% w/v, 2 drops) were added to the FAMEs and allowed to stand at room temperature overnight. The solution was then quenched with aqueous sodium thiosulphate (5% w/v, 500 µl) and the DMDS derivatives extracted with hexane (3 x 1 ml). The extracts were combined and used directly for GC-MS analysis. Quantification was carried out manually using relative abundance of the major fragment ions.

2.5.5 Trimethylsilylation

Lipid extracts were blown down and BSTFA was added (*N,O*-bis (trimethylsilyl) trifluoroacetamide containing 1% v/v trimethylchlorosilane, 20 µl). The mixture was heated to 70° C for 60 min. The resulting trimethylsilyl (TMS) derivatives were dissolved in the appropriate amount of hexane and submitted to GC and GC/MS.

2.6 Instrumental analysis

2.6.1 Gas chromatography (GC)

The GC analyses were performed on a Hewlett Packard 5890 gas chromatograph coupled to an Opus V PC using HP Chemstation software, which provided instrument control, data acquisition and post-run data processing facilities. Total lipid extracts were

introduced by on-column injection into a 15 m x 0.32 mm i.d. WCOT fused silica capillary, coated with DB1 stationary phase (immobilised dimethyl polysiloxane, 0.1 μm film thickness). The temperature programme consisted of a 2 min isothermal hold at 50°C followed by a ramp from 50 to 350° C at 10° C min^{-1}. The temperature was then held at 350° C for 10 min. Samples prepared as their methyl esters, TMS ethers or as their DMDS derivatives were analysed using a 50 m x 0.32 mm i.d. WCOT fused silica capillary, coated with a 100% polymethylsiloxane stationary phase (CPSil-5 CB, 0.12 μm film thickness). The temperature program consisted of two ramps, first from 50 to 200° C at 10° C min^{-1}, then from 200 to 300° C at 4° C min^{-1}. The temperature was then held at 300° C for 10 min. Samples prepared as their methyl esters were analysed using a 25 m x 0.32 mm i.d. WCOT fused silica capillary, coated with a polyethylene glycol stationary phase (CP-Wax-52CB, 0.2 μm film thickness). The temperature program consisted of a 2 min isothermal hold at 50° C followed by 2 ramps from 50 to 170° C at 10° C min^{-1}, then from 170 to 220° C at 2° C min^{-1}. The temperature was then held at 220° C for 10 min. In all cases, hydrogen was used as carrier gas (head pressure 10 psi) and flame ionisation detection used to monitor the column effluent.

Quantification of the different components of the lipid extracts was performed by comparing the area under each peak to the area under the internal standard peak. Reproducibility of the experimental decay and of the extraction procedure was assessed using replicate samples at all time points during the experimental decay of dairy fats, and was estimated to be 5% RSD. Reproducibility of the analysis by high temperature gas chromatography was assessed using regular injection of a standard mixture containing 3 fatty acids, 2 monoacylglycerols, cholesterol, n-triatetracontane, 2 diacylglycerols and 3 triacylglycerols and was estimated to be 2% RSD. Injection of this standard mixture also enabled the monitoring of the performance of the HTGC system (Figure 2.4).

2.6.2 Gas chromatography-mass spectrometry (GC-MS)

GC/MS analyses were performed using a Finnigan 4500 quadrupole mass spectrometer (source temperature, 280° C; filament current, 0.35 mA; electron voltage, 35 eV) interfaced to a Carlos Erba HRGC 5160 Mega series gas chromatograph. The GC operating conditions were the same as described above except that helium was used as the carrier gas. Data were acquired and processed using an INCOS data system.

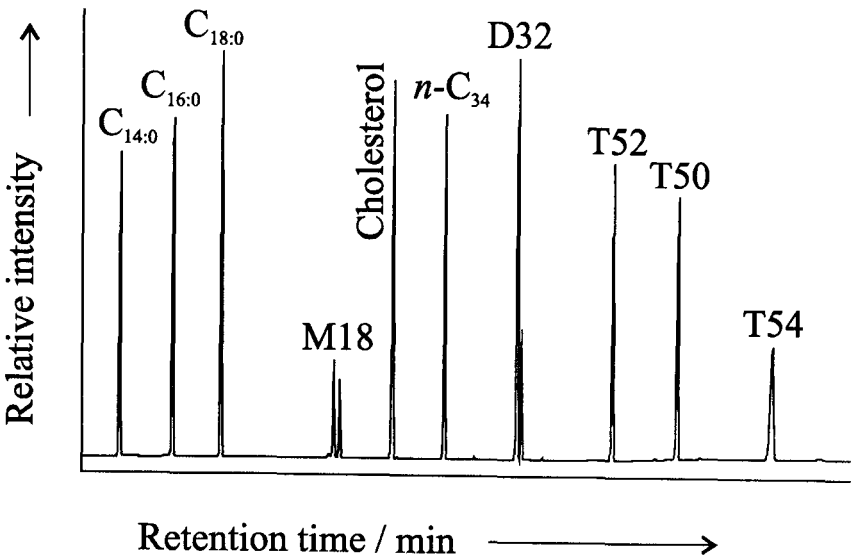


Figure 2:4: Partial HTGC trace of the trimethylsilylated standard mixture used to monitor the performance of the gas chromatographs fitted with a high temperature column (DB1). Peak identies: $C_{m:n}$ = free fatty acids with m carbon atoms and n double-bonds; M18 = monoacyglycerol with 18 acylcarbon atoms; *n*-C_{34} = *n*-tetratriacontane; D32 = diacylglycerol with 32 acyl carbon atoms; T50 – T54: triacylglycerols with 50 to 54 acyl carbon atoms.

2.6.3 Gas chromatography-combustion-isotope ratio-mass spectrometry (GC-C-IRMS)

GC-C-IRMS analyses were performed using a Varian 3400 gas chromatograph attached to a Finnigan MAT Delta S mass spectrometer *via* a modified Finnigan MAT Mark I combustion interface with a copper oxide and platinum catalyst maintained at 650° C. A Nafion membrane prevented water from reaching the ion source. The mass spectrometer source pressure was 6.10^{-6} mbar. Samples were analysed using a using a 50 m x 0.32 mm i.d. WCOT fused silica capillary, coated with a 100 % polymethylsiloxane stationary phase (CPSil-5 CB, 0.12 μm film thickness). Helium was used as carrier gas. Carbon isotope ratios were expressed relative to VPDB, $\delta^{13}C$ / ‰ = $1000[(R_{sample}-R_{standard})/R_{standard}]$, where R is $^{13}C/^{12}C$.

2.7 Soil analysis

2.7.1 pH

Soil pH was measured by using a soil suspension in water (10 g of dry soil in 25 ml deionized water). The suspension was allowed to stand with occasional mixing for 30 min. Three pH readings of the surnatant solution was taken using a hand held pH-meter (pH tester Checker 1, Hanna Instruments).

2.7.2 Cation Exchange Capacity (CEC)

Exchangeable cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) were extracted from soil samples using ammonium ethanoate buffer (1 M, pH 7). A 1:4 soil suspension (5 g dry soil in 20 ml extractant) was left to stand overnight. The solutions were then separated from the soils by filtration through filter paper (Whatman N.1) into volumetric flasks (100 ml), the soil being thoroughly rinsed with ammonium ethanoate solution. This extract was used to measure exchangeable cations by atomic absorption spectroscopy (AAS) using a Pye Unicam SP9 atomic absorption spectrometer linked to a Pye Unicam SP9 computer for data collection. The instrumental conditions are given in the Table 2.5. The CEC at pH 7 was determined by the sum of the exchangeable cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) and was expressed in $cmol_c\ kg^{-1}$ (centimoles of charge per kg of dry soil, expressing the number of negative sites able to retain a positive cation)

Table 2.5: Instrumental conditions used during cation analysis by AAS.

Cation	Flame type	Principal line / nm	Lamp current / mA
Ca^{2+}*	Lean-stoichiometric air/acetylene	422.7	4
Mg^{2+}*	Lean-stoichiometric air/acetylene	285.2	2
K^+	Stoichiometric air/acetylene	766.5	5
Na^{2+}	Lean-stoichiometric air/acetylene	589.0	5

*Lanthanum, as the chloride, at the final concentration of 800 mg ml^{-1}, was used as a releasing agent in the analysis of calcium and magnesium.

2.7.3 Particle size distribution

Soil samples were sieved into 2 fractions, > 250 μm and < 250 μm, and the smaller fraction was submitted to particle size analysis (Mastersizer μ Ver 2.12, Malvern Instruments). Results were expressed as volume percentage of the different fractions, as described in the Table 2.6.

Table 2.6: Particle size of the fractions used for soil structural class assignment.

Fraction	Particle size range / μm
Coarse sand	200 - 2000
Fine sand	60 - 200
Silt	2 -60
Clay	< 2

Soil structural classes were assigned using the UK soil textural classes (Rowell, 1994)

2.8 Elemental analysis

Elemental analysis was performed on dry soil samples before solvent extraction and on ground potsherds before and after solvent extraction using a Perkin Elmer 240 C elemental analyser. The mass percentage of carbon, nitrogen, hydrogen and phosphorus were determined before solvent extraction. The percentage of inorganic carbon (carbonate) was also measured and subtracted from the total carbon content to obtain the mass percentage of organic carbon.

CHAPTER 3: PHYSICOCHEMICAL ALTERATIONS OF LIPID RESIDUES DURING VESSEL USE AND/OR BURIAL

3.1 Introduction and aims of the chapter

The high porosity of fired ceramics allows the walls of unglazed vessels to absorb the lipid components of vegetable and animal commodities processed in the vessel (Charters, 1996; Oetgen, 1983), and it is also likely that absorption of soil lipids will occur during vessel burial, which would alter the composition of any residue resulting from vessel use. However, Heron and co-workers (1991) investigated the contribution of soil lipids towards absorbed residues by comparing the HTGC profiles of the total lipid extracts from potsherds and their immediate burial sediments, and concluded that no significant migration of lipids from the burial environment to the potsherds takes place during vessel burial.

The majority of lipid residues associated with archaeological potsherds derive from animal fats or vegetable waxes and oils, and consists of free fatty acids, acylglycerols, sterols, wax esters, alkanes and alcohols (Charters, 1996; Dudd, 1999; Bland 1999). Oxidation products such as α,ω-dicarboxylic acids and hydroxy acids have also been identified in vessels excavated from arid sites (Regert *et al.*, 1998; Bland, 1999). Lipids, by definition are hydrophobic which enables them to remain associated with potsherds during vessel burial, unlike proteins and sugars (Rottländer, 1990). Short-chain components are however known to be more water-soluble than their longer-chain counterparts (Dils, 1983) and oxidation products such as those cited above are thought to be more water-soluble than their fatty acid precursors (Regert *et al.*, 1998). The absence of oxidation products in the solvent-extractable fraction associated with archaeological potsherds has been related to their relatively higher water-solubility making them susceptible to leaching into groundwater (Regert *et al.*, 1998).

The aims of this chapter are:

(i) To investigate the absorption of lipids by unglazed ceramic potsherds during burial and dosage.

(ii) To assess the contribution of soil lipids to absorbed residues by studying lipid residues from two assemblages of unglazed ceramic potsherds and their immediate burial soils.

3.2 Lipid absorption

3.2.1 Absorption of olive oil into unglazed ceramics

Potsherds were dosed in solutions of olive oil in dichloromethane of increasing concentration by sonication for various times in order to determine the optimum conditions for potsherds dosage for experimental burial (Section 2.2.2.1). Potsherds were extracted as described in Section 2.5.1 to yield the TLE which was derivatised (Section 2.5.5) and analysed by HTGC (Section 2.6.1).

3.2.1.1 Influence of the dosage time

Potsherds were sonicated in olive oil solution (100 mg ml^{-1} in dichloromethane) for up to 120 min. The potsherd lipid content (Figure 3.1) strongly increased during the first 20 min of sonication (from 0 to 0.9 mg g^{-1}), and remained constant thereafter. Based on these observations, experimental potsherds intended for decay experiments were dosed twice for 15 min. An initial strong absorption of various foodstuffs, including olive oil, followed by consecutively smaller increments has been previously observed (Oetgen, 1983) during the dosage of blank potsherds (sonication was not used in these experiments), and is thought to be due to some sealant property of the foodstuff itself. This sealant effect of animal fats absorbed in unglazed ceramics has also been reported by Charters (1996) during the experimental cooking of lamb meat.

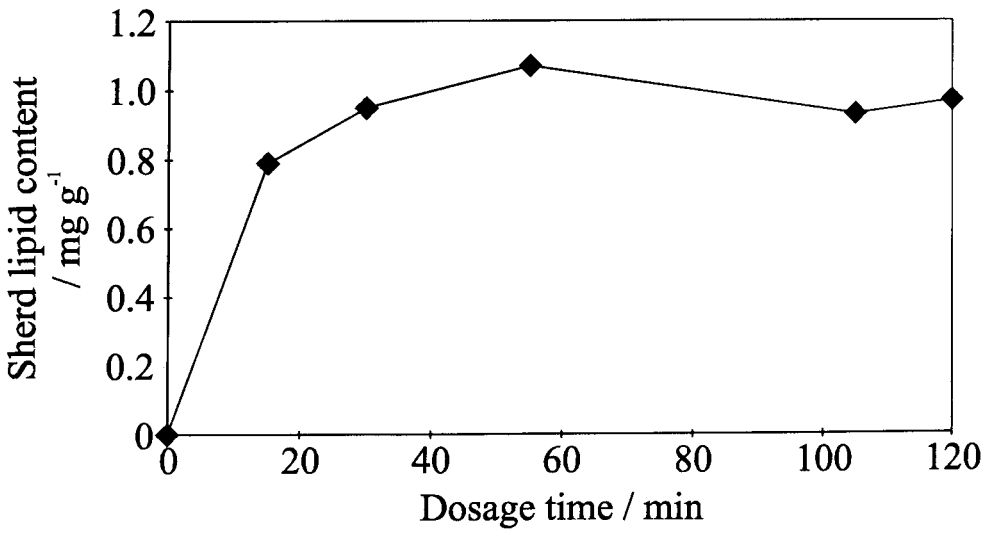


Figure 3.1: Changes in the potsherd lipid content with increasing sonication time (potsherds were dosed in a 100 mg ml^{-1} olive oil solution in dichloromethane).

3.2.1.2 Influence of the concentration of the olive oil solution

Potsherds were dosed twice for 15 min in olive oil solutions in dichloromethane (0-500 mg ml^{-1}) and pure olive oil. Figure 3.2 shows that the potsherd lipid content increased linearly with the concentration of the olive oil solution (from 0 to 133 mg g^{-1}). It was decided to dose potsherds aimed at experimental decay in 100 mg g^{-1} solutions, as the lipid absorbed within the potsherd was at the concentration deemed suitable for experimental purposes.

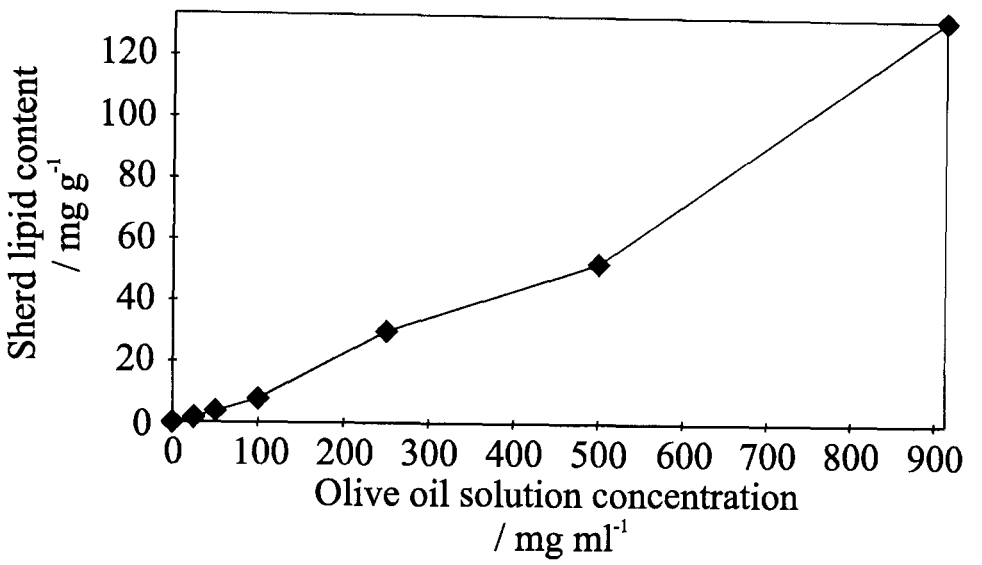


Figure 3.2: Changes in the potsherd lipid content with increasing olive oil concentration (potsherds were dosed for 2 x 15 min).

3.2.1.3 Influence of the potsherd mass

Potsherds of increasing mass were dosed in an olive oil solution (100 mg ml^{-1}) for 2 x 15 min. Figure 3.3a shows that there is a linear relationship between the potsherd mass and the potsherd total lipid content, expressed in mg. However, Figure 3.3b shows that there is no clear relationship between the potsherd mass and the potsherd lipid content, expressed in mg g^{-1}. When the potsherd mass was increased from 2 to 6 g, the potsherd lipid content varied between 2.3 and 3.8 mg g^{-1}. Above a potsherd mass of 6 g, the potsherd lipid content is relatively constant at *ca.* 6.5 mg g^{-1}. It is very likely that the amount of lipid absorbed within the ceramic matrix is not governed by the potsherd mass but rather by the potsherd surface area which does not vary significantly for the heavier potsherds used in this experiment.

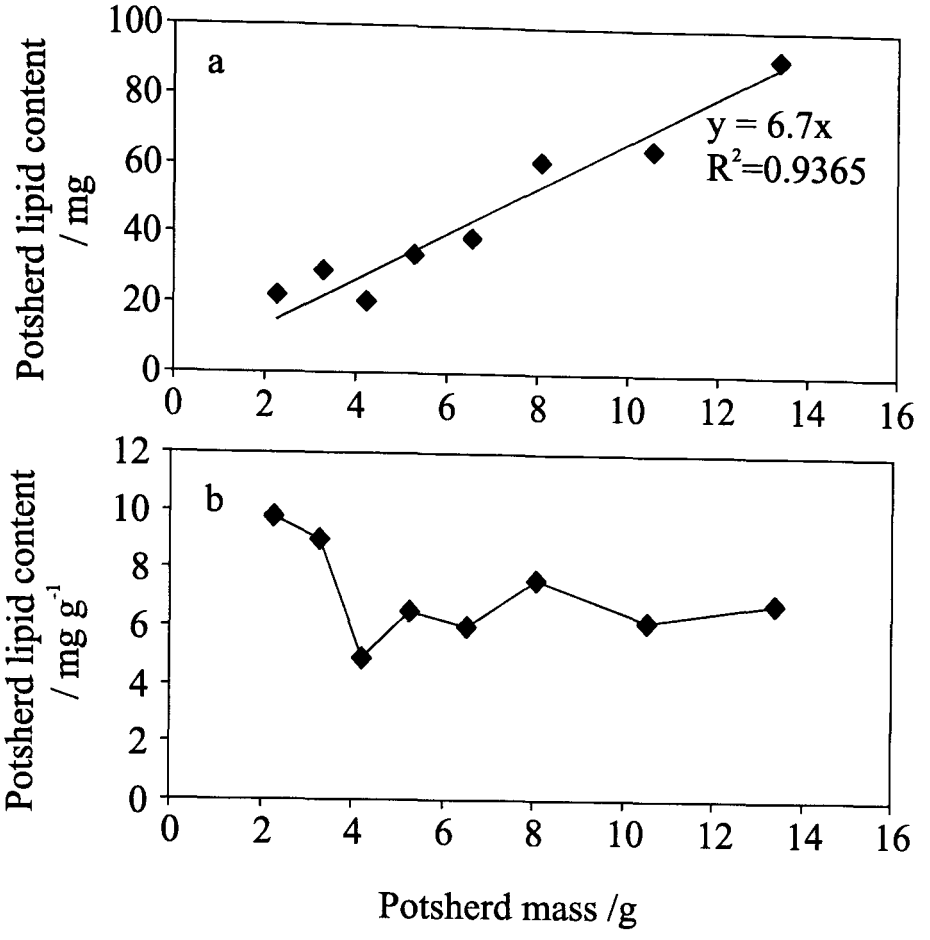


Figure 3.3: Changes in the potsherd lipid content, expressed in a) mg, and b) mg g^{-1}, with increasing potsherd mass (potsherds were dosed in a 100 mg ml^{-1} olive oil solution in dichloromethane for 2 x 15 min).

3.2.2 Absorption of lipids from mushroom compost

Two experiments were set up in which blank potsherds were buried in mushroom compost to investigate the absorption of lipids from the burial environment by unglazed potsherds (Section 2.3.1). After 2 years of incubation, potsherds from these experiments did not contain significant amounts of lipids (< 5 μg g^{-1} lipid for both experiments) and it was not possible to identify the compounds present due to their low abundance. This is consistent with the fact that: (i) there is a high incidence of blank potsherds in the archaeological record (Berstan and Evershed, personal communication), and (ii) Heron and coworkers (1991) did not find any similarities between the HTGC profiles of archaeological potsherds and those of their embedding sediments. Together these observations confirm that the contribution of lipids in absorbed lipid residues is insignificant.

3.3 Archaeological samples and burial soils

Unglazed ceramic potsherds and their burial sediments were sampled from 2 archaeological sites as described in the Section 2.1.1 in order to assess the contribution of

soil lipids to absorbed residues. Tables 3.1 and 3.2 show the sample names.

Table 3.1: Potsherds and burial sediments sampled in Eton.

Pit	Context	Potsherds	Burial sediments
2102	2100	2102/1	Sed-1
2102	2101	2102/2/1	Sed-2
2102	2101	2102/2/2	Sed-2
2102	2101	2102/3	Sed-3
2102	2101	2102/5	Sed-5
2102	2100	2102/6	Sed-6
2102	2101	2102/8/1	Sed-8
2102	2101	2102/8/2	Sed-8
2102	2100	2102/15	Sed-15
668	-	668/3	Sed-3b
668	-	668/4	Sed-4b
668	-	668/5	Sed-5b

Table 3.2: Potsherds and burial sediments sampled in Crick.

Potsherds	Burial sediments
Crick-A	Sed-A
Crick-B	Sed-B
Crick-C	Sed-C
Crick-D1	Sed-D
Crick-D2	Sed-D
Crick-D3	Sed-D
Crick-D4	Sed-D
Crick-E	Sed-E
Crick-F	Sed-F
Crick-G	Sed-G
Crick-H	Sed-H
Crick-I	Sed-I
Crick-J1	Sed-J
Crick-J2	Sed-J
Crick-K	Sed-K
Crick-L	Sed-L

3.3.1 Eton

3.3.1.1 Total lipid extracts

(i) Potsherds

The total lipid content of the potsherds from Eton, shown in Figure 3.4, ranged between < 5 to 222 $\mu g\ g^{-1}$. The 3 potsherds from pit 668 contained less than 5 $\mu g\ g^{-1}$, whereas 5

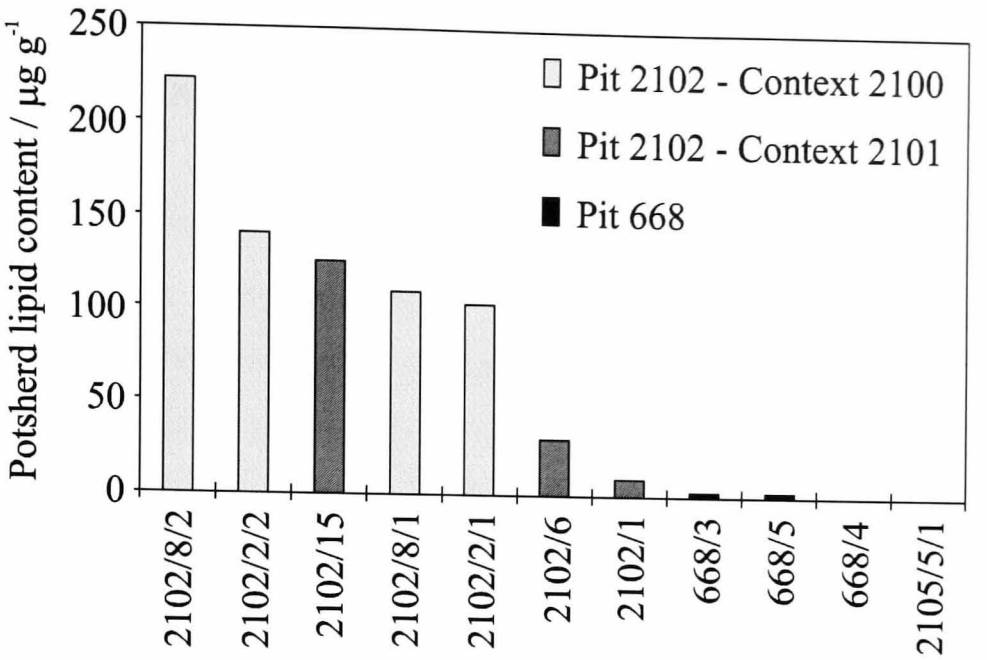


Figure 3.4: Total lipid content in the potsherds from Eton.

from the 9 potsherds from pit 2102 contained more than 100 μg g^{-1} lipid. It was therefore decided to focus investigation on the 6 potsherds from pit 2101 having the highest lipid content.

The HTGC traces of the total lipid extracts from these 7 potsherds were remarkably similar. Figure 3.5 shows the partial HTGC trace of the residues from the potsherd 2102/2/2 as an example. The preservation of the residue was very good as it still contained a high abundance of triacylglycerols, ranging from C_{42} to C_{54}. Partial hydrolysis had taken place during vessel use and/or burial, as demonstrated by the presence of diacylglycerols, monoacylglycerols and free fatty acids. The free fatty acid distribution was dominated by $C_{16:0}$, and $C_{18:0}$, with the unsaturated fatty acid $C_{18:1}$ and the medium-chain $C_{14:0}$ present in lower abundance. The branched odd-numbered $C_{15:0}$ and $C_{17:0}$ fatty acids were also identified. This fatty acid distribution is consistent with an animal fat origin for the residue (Dudd, 1999). The presence of branched odd-numbered fatty acids is characteristic of degraded ruminant fats (Dudd, 1999), as such compounds are produced in the rumen by the action of microorganisms (Gunstone *et al.*, 1986). Dudd and coworkers (1998) have observed the incorporation of such compounds during the experimental decay of olive oil and pure triacylglycerols absorbed in unglazed ceramics, albeit in minor quantities. Hence, while these compounds could have arisen from microbial activity on the surface of the potsherd, the residues recovered from Eton were otherwise characteristic of ruminant fats. Furthermore, the wide carbon number ranges of mono- (C_{14} to C_{16}), di- (C_{30} to C_{34}) and triacylglycerols (ranging from C_{40} to C_{54}; shown in Figure 3.6), and the presence of $C_{14:0}$, is characteristic of degraded dairy fat

(Dudd, 1999).; confirmation of these assignments requires study of the stable carbon isotope ratios of the $C_{16:0}$ and $C_{18:0}$ fatty acids from these residues (Section 3.3.1.2).

The HTGC traces of all the potsherds excavated from pit 2102 revealed the same components. The only difference between the potsherds, apart from the total lipid content, was the relative abundance of the intact triacylglycerols and free fatty acids relative abundance. Figure 3.7 shows the free fatty acid to triacylglycerol ratio, which can be considered to be an indicator of the extent of degradation as it mainly reflects ester hydrolysis of the triacylglycerols. Loss of free fatty acids from the potsherds by solubilisation into groundwater also affects this ratio, giving an impression of enhanced triacylglycerol preservation. However, the wide range of free fatty acid to triacylglycerol ratios for the residues recovered from Eton [from 0 (for residues which showed free fatty acids) to *ca.* 1 (for residues containing equivalent amounts of triacylglycerols and free fatty acids)] infers that these residues have been affected by degradation to very different extents. This observation was unexpected as these residues have been buried under similar burial conditions for similar periods of time.

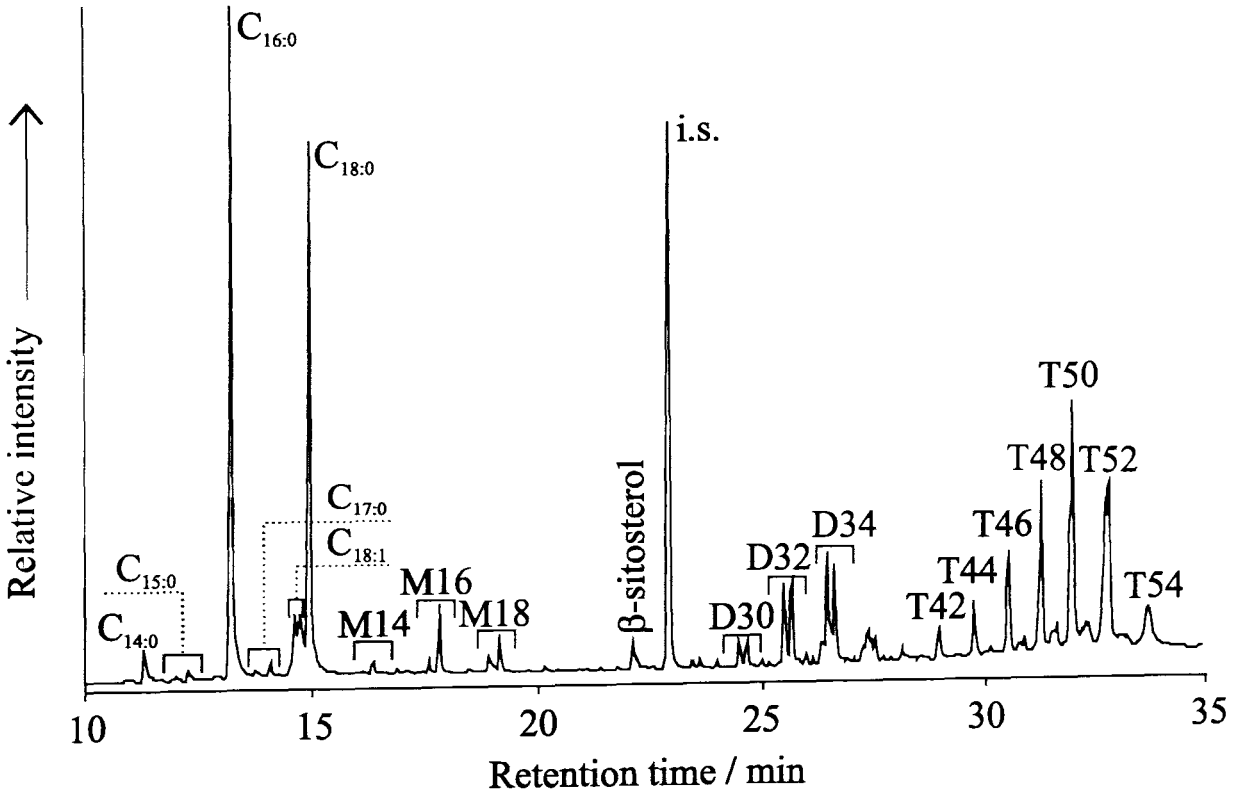


Figure 3.5: Partial HTGC trace of the trimethylsilylated total lipid extract from the potsherd 2102/2/2. Peak identities: $C_{m:n}$ = free fatty acid with m carbon atoms and n double-bonds; M14-M18 = monoacylglycerols with 16 to 18 acyl carbon atoms; i.s. = internal standard (*n*-tetratriacontane); D30-D34 = diacylglycerols with 30 to 34 acyl carbon atoms; T42-T54 = triacylglycerols with 42 to 54 acyl carbon atoms.

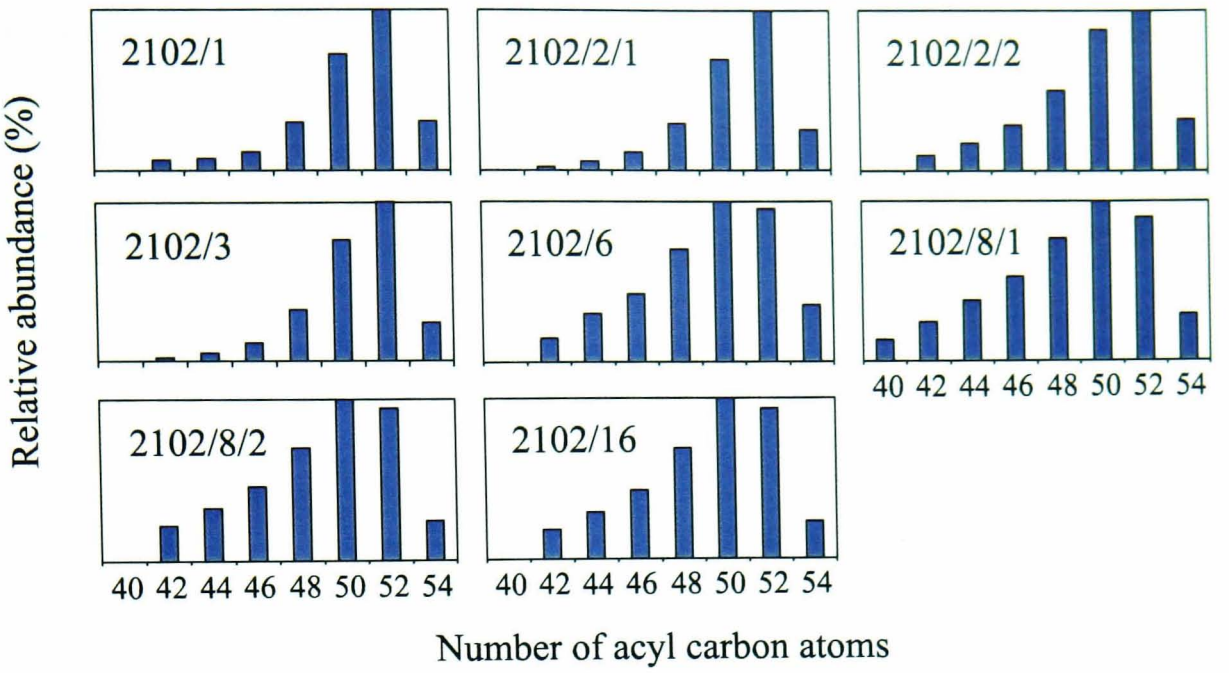


Figure 3.6: Triacylglycerol distribution in the total lipid extracts from the potsherds from Eton.

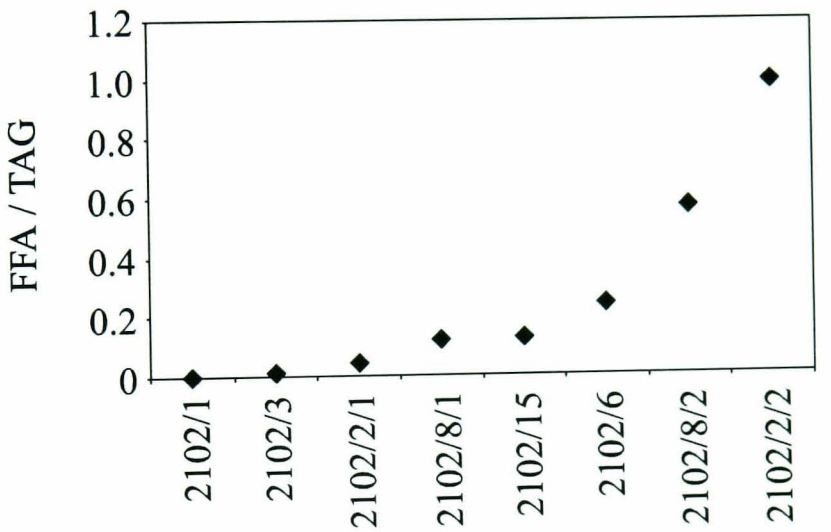


Figure 3.7: Free fatty acid (FFA) to triacylglycerol (TAG) ratio for the potsherds from Eton.

(ii) Burial soils

All soils displayed pHs ranging between 7.1 and 7.9 and were identified as sandy loam, with a clay content ranging between 2.2 and 4.3 % (see Section 2.7.3). Other properties of the burial soils, including CEC, organic carbon and carbonate content, are summarised in Table 3.3. The C/N ratio of the burial soils ranged between 4.8 and 21.0. The C/N ratio of living plant tissues ranges between 18 and 47 for soft parts (leaves) and between 32 and 99 for hard tissues (stem; Lallier-Verges *et al.*, 1998). Soil organic matter degradation leads to preferential loss of carbon (evolved as CO_2 or CH_4) while N is fixed in the sediment (absorbed as NH_4^+ onto clay surface, or included in resistant molecular moieties within the structures of humic substances) and thereby, to lower C/N ratios.

Both C/N and C/P are in the range required to sustain the soil microorganisms involved in organic matter decomposition (Gray, 1989).

Burial soils were soxhlet-extracted (Section 2.4.2.2) to yield the total lipid extract that was derivatised (Section 2.5.5) and submitted to HTGC (Section 2.6.1). Table 3.4 shows the average lipid content for the 2 contexts in pit 2101.

Table 3.4: Average total lipid content of soils in the contexts 2100 and 2101 at Eton.

Context	Lipid content / $\mu g\ g^{-1}$
2100	14 ± 5[1]
2101	12 ± 5[2]

[1] $n = 3$
[2] $n = 5$

All HTGC traces were very similar and the HTGC trace of the residue from the soil Sed-2 is shown in Figure 3.8 as an example. It was dominated by fatty acids, ranging from $C_{16:0}$ to $C_{34:0}$, and also contained a series of *n*-alcohols and fatty acids. Given the importance of the study of the stable carbon isotope ratios of individual fatty acids in the identification of the origin of archaeological residues, it was decided to concentrate this investigation on the total fatty acid fraction of the burial soils.

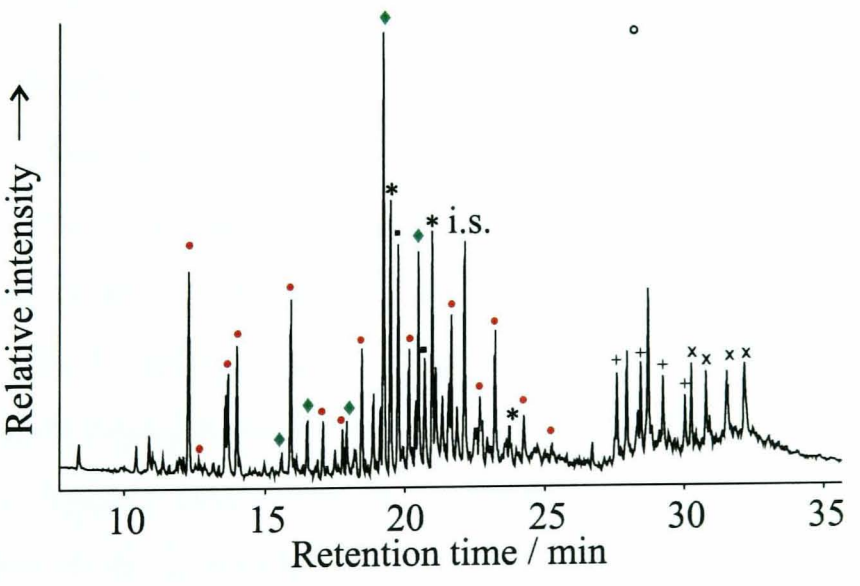


Figure 3.8: Partial HTGC trace of the trimethylsilylated total lipid extract from the burial soil Sed-2. Peak identities: ´ = fatty acids; ■ = *n*-alkanes; ◆ = fatty alcohol; * = ω-hydroxy fatty acids; + = wax esters; x = triacylglycerols.

3.3.1.2 Fatty acid fractions

(i) Potsherds

Total lipid extracts were saponified (Section 2.5.2) and methylated (Section 2.5.3) prior to GC analysis on a polar column (Section 2.6.1). The total fatty acid distributions are summarised in Table 3.5.

Table 3.5: Fatty acid distribution in the total lipid extract from the potsherds from Eton.

	Relative abundance (%)							
	2102/1	2102/2/1	2102/2/2	2102/3	2102/6	2102/8/1	2102/8/2	2102/15
Compound								
$C_{12:0}$	0	0	0.3	0	0	1.2	0.5	1.1
$C_{14:0}$	6.8	14.1	9.2	4.3	15.2	14.2	10.1	12.9
$C_{16:0}$	49.4	63.5	53.6	49.9	58.8	49.6	46.9	50.5
$C_{18:0}$	38.6	22.4	30.9	37.9	26.0	27.2	30.8	28.0
$C_{18:1}$	5.1	0	6.0	7.9	0	7.8	11.7	7.5

Figure 3.9 shows the relative abundance of the $C_{18:1}$ isomers in the residues extracted from the potsherds from Eton. These residues have tentatively been identified as degraded ruminant fats based on their HTGC profiles and on their total fatty acid distribution. In ruminant fats, oleic acid (*cis*-$C_{18:1}\Delta^9$) is the most abundant $C_{18:1}$ isomer and microorganisms in the rumen characteristically produce other isomers *via* biohydrogenation including the *trans* Δ^9, *trans* Δ^{10}, *trans* Δ^{11} and *trans* Δ^{13} isomers (Gunstone *et al.*, 1986). Oleic acid was the most abundant isomer in all potsherd residues, except 2108/1, in which *trans*-$C_{18:1}\Delta^9$ was the most abundant isomer. All residues resembled degraded ruminant fats (see Section 5.2) but showed reduced abundance of *cis*-$C_{18:1}\Delta^9$ and increased abundance of *trans*-$C_{18:1}\Delta^9$ and *trans*-$C_{18:1}\Delta^{11}$. Similar altered compositions have been reported in degraded animal fats absorbed in unglazed ceramics (Dudd, 1999). The *trans* configuration is the thermodynamically preferred configuration of double-bonds (Morrison and Boyd, 1992) and unsaturated fatty acid with double-bonds in the *trans* configuration identified in sediments are thought to originate from direct microbial input or from microbial modification of pre-existing sedimentary fatty acids, or even from clay-catalysed isomerisation (Stefanova and Disnar, 2000). It is also possible that unsaturated fatty acids containing double-bonds with the *trans* configuration are not degraded at the same rate by microorganisms, resulting in the preferential accumulation of the more stable isomer (Lewis *et al.*, 1999)

Table 3.3: Physico-chemical properties of the burial soils in Eton.

Sample	Context	pH	CEC / $cmol_c\ kg^{-1}$	Organic carbon content (%)	C/N	C/P	Carbonate content (%)	Clay content (%)	Textural class
Sed-1	2101	7.6	291	0.4	5.9	2.6	1.2	3.0	Sandy loam
Sed-2	2100	7.5	366	1.4	17.0	13.1	0.8	3.9	Sandy loam
Sed-3	2101	7.9	508	0.8	15.3	4.3	1.2	3.2	Sandy loam
Sed-5	2100	7.7	321	0.9	11.5	7.1	0.7	2.2	Sandy loam
Sed-6	2100	7.5	453	1.0	17.8	7.5	0.4	3.2	Sandy loam
Sed-8	2100	7.1	238	0.5	4.8	3.2	0.1	3.5	Sandy loam
Sed-15	2100	7.5	779	0.5	21.0	10.5	0.5	4.3	Sandy loam

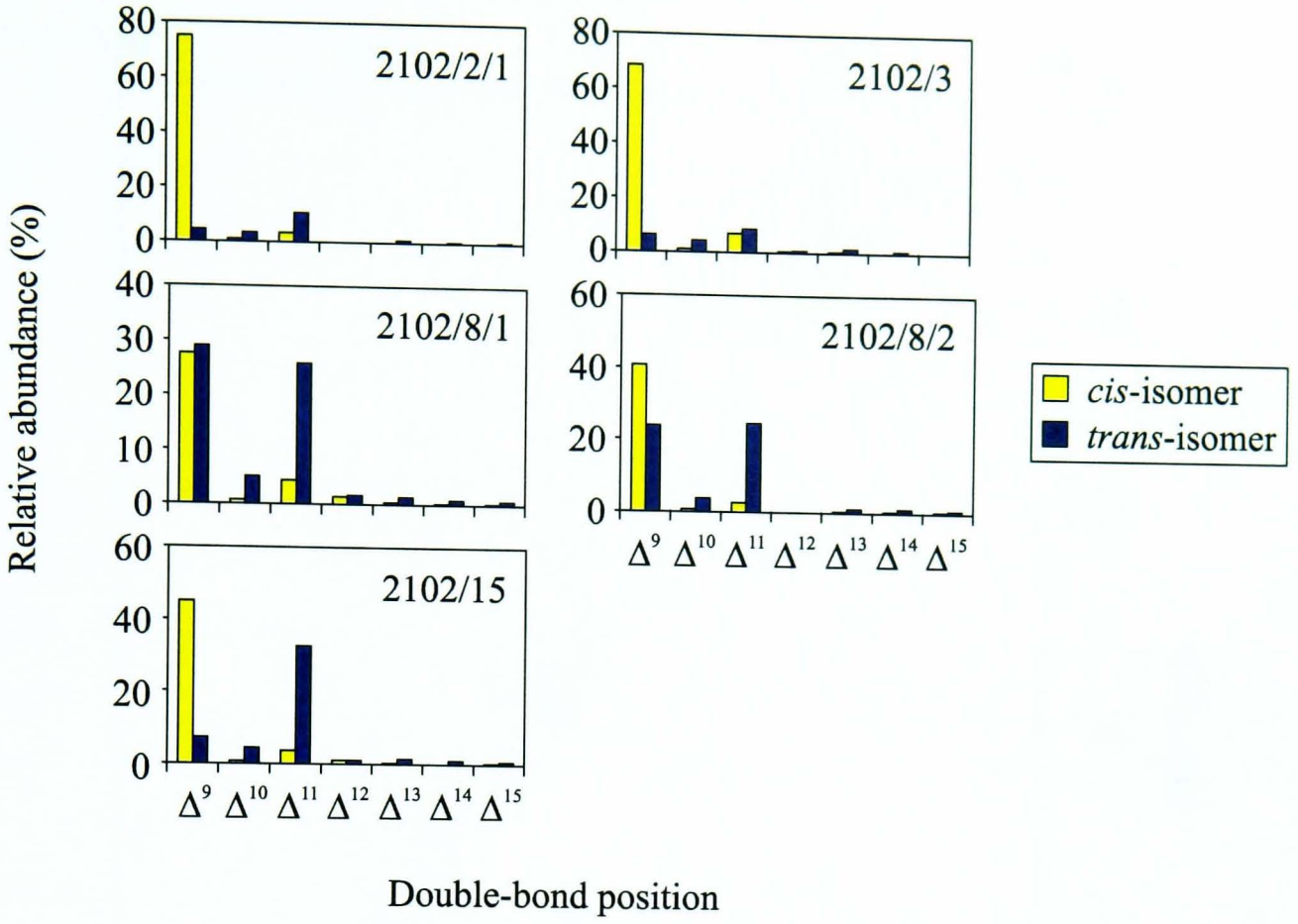


Figure 3.9: Distributions of the $C_{18:1}$ isomers in the potsherds from Eton.

Figure 3.10 shows $\delta^{13}C$ values for $C_{16:0}$ and $C_{18:0}$ for the potsherds excavated from Eton, compared with the values for some reference fats (Dudd, 1999). The values for the potsherds 2102/8/1, 2102/8/2, 2102/2/2 and 2102/15 were clustered around $\delta^{13}C_{16:0}$ = -28 ‰ and $\delta^{13}C_{18:0}$ = -32 ‰. The potsherd 2102/2/1 exhibited a similar value for $C_{16:0}$, but $C_{18:0}$ was more enriched at –29.5 ‰. The fatty acids from the potsherd 2102/3 were the most enriched, with $\delta^{13}C_{16:0}$ = -27 ‰ and $\delta^{13}C_{18:0}$ = -29 ‰. The values of $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ for the potsherds 2102/8/1, 2102/8/2, 2102/2/2 and 2102/15 were not really characteristic of any of the reference fats presented here. This may be due to the lipid residues also containing plant constituents, due to mixing or sequential processing of different commodities in the vessels. The values for 2102/8/1 and 2102/8/2 were very similar and visual inspection of the potsherds revealed that they belonged to the same vessel. The isotope values for 2102/2/1 and 2102/3 are consistent with the values for the mixing curve between lamb and pig fat. This could indicate a simultaneous or sequential processing of pig and lamb products in these vessels.

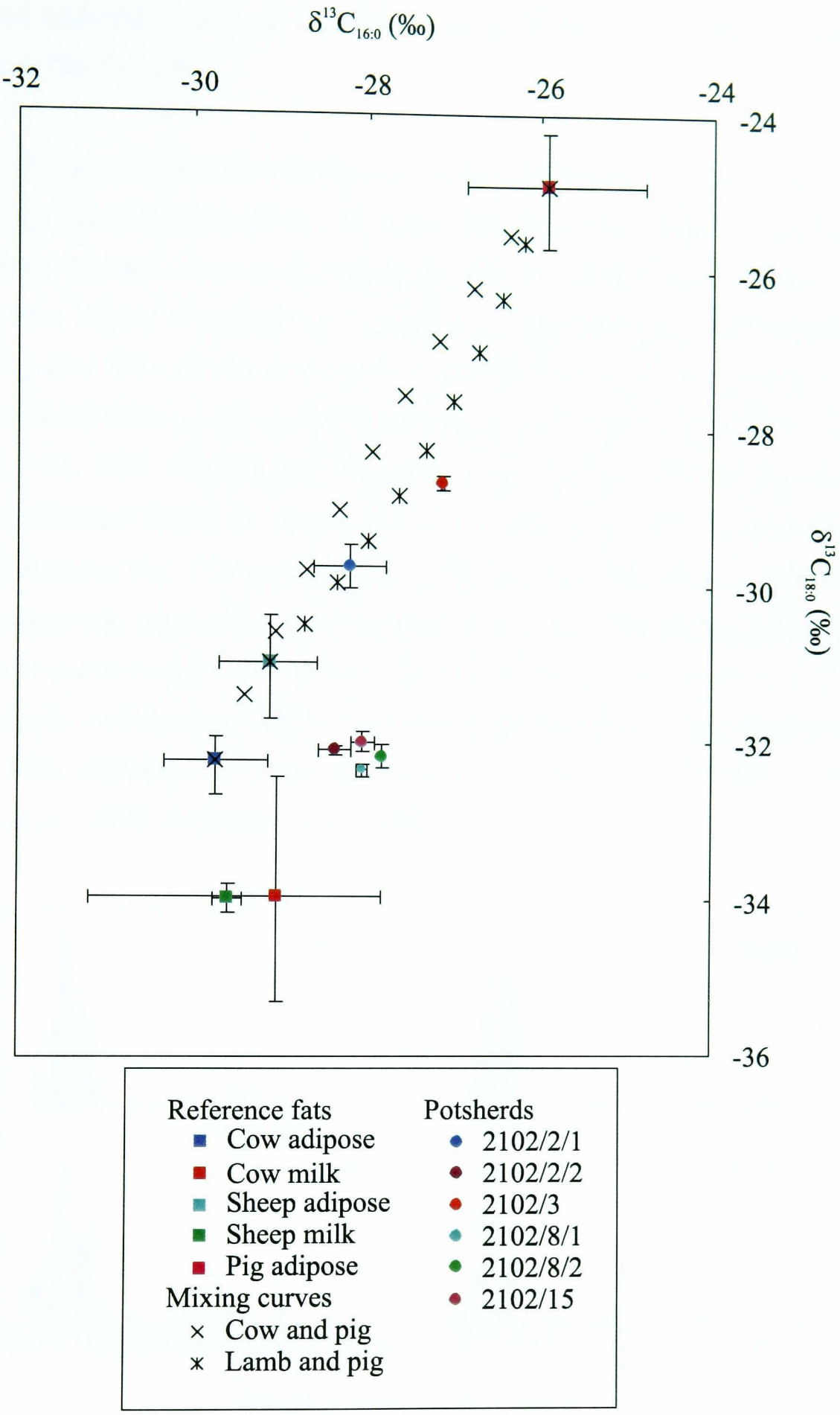


Figure 3.10: $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in the potsherds from Eton, compared to reference fats (Dudd, 1999).

(ii) Burial soils

An aliquot of the total lipid extract was saponified (Section 2.5.2) prior to acid/neutral separation (Section 2.5.1) to yield the acid fraction, which was methylated (Section

2.5.3) and analysed by GC on a CPSil-5 column to allow the elution of long-chain compounds (Section 2.6.1).

Figure 3.11 shows the fatty acid distribution of the burial sediments from Eton, compared to the fatty acid distribution for the potsherds. The fatty acid distributions were significantly different between the potsherds and the embedding sediments. All soil samples were largely dominated by $C_{18:0}$ and $C_{16:0}$, and contained saturated fatty acids containing up to 30 or 32 carbon atoms, with predominance of the fatty acids containing even-numbered carbon atoms. Similar distributions are commonly observed in sediments (Amblès *et al.*, 1994, Naraoka and Ishiwatari, 2000, Stefanova and Disnar, 2000). The Carbon Preference Index for the longer chain fatty acids (CPI = 2Σ(even C_{20} to C_{28})/[Σ(odd C_{19} to C_{27}) + Σ(odd C_{21} to C_{29})], which is a measure of the predominance of the even-numbered fatty acids over the odd-numbered ones, was calculated to be between 5.3 (Sed-2) and 9.2 (sed-15), which is less than the value for the leaves of C3 plants (>10; Naraoka and Ishiwatari, 2000). The longer chain fatty acids are thought to originate directly from vegetation, or from the oxidation of plant-derived alkanes or alkanols (Amblès *et al.*, 1994; van Bergen *et al.*, 1998).

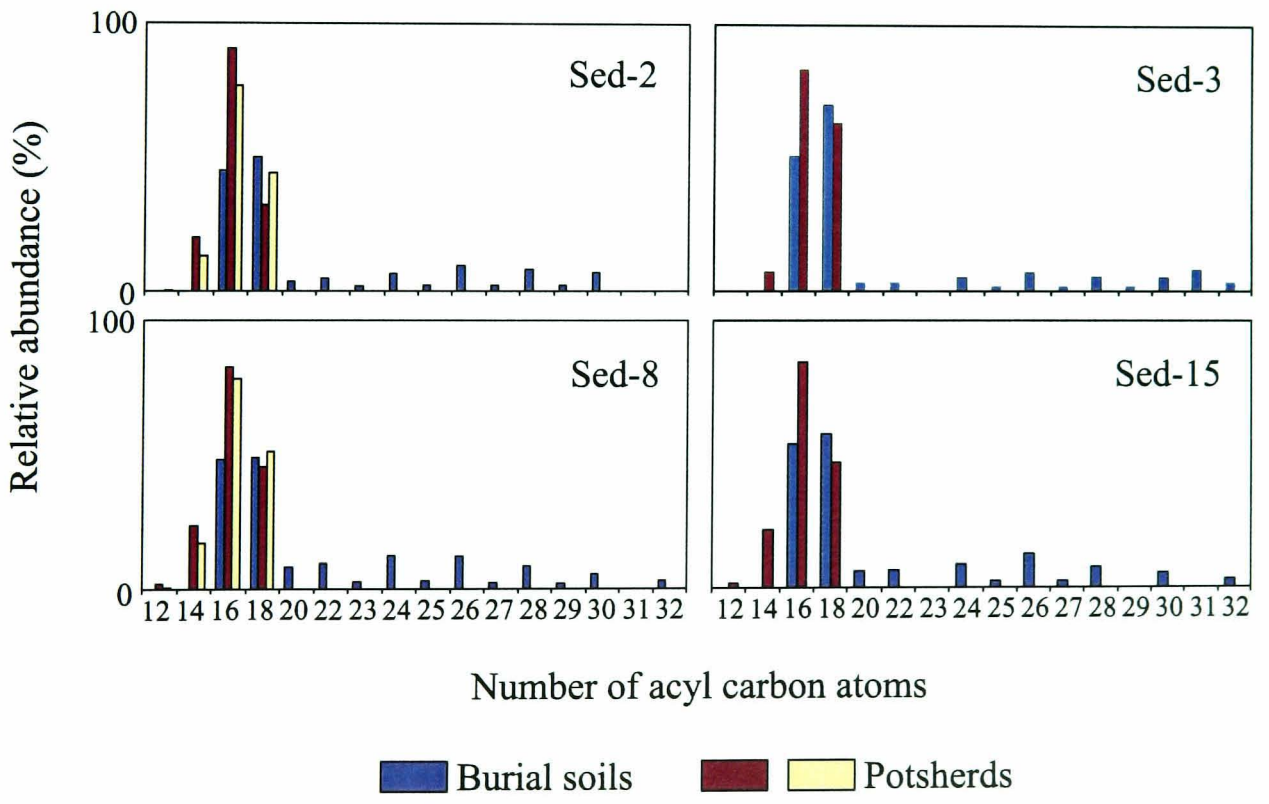


Figure 3.11: Fatty acid distribution in the total lipid extracts from the potsherds and burial soils from Eton.

Table 3.8 shows the $\delta^{13}C$ values for individual fatty acids from the burial soils. All samples exhibited a $\delta^{13}C_{16:0}$ of around –26.7 ‰, except Sed-3 for which the value was –22.3 ‰. The $C_{18:0}$ fatty acid was more enriched, with values around –24.6 to –25.1 ‰, the value for Sed-3 being again more enriched at –19.1 ‰. The longer-chain fatty acids exhibited more depleted values in all samples, ranging from –28.7 to –36.0 ‰. Identical values have been reported by Lichtfouse and coworkers (1995) who argued that the difference in isotopic value between $C_{16:0}$ and $C_{18:0}$, and the longer-chain fatty acids reflected the different origin of these compounds. The longer-chain fatty acids were found to originate from plant compounds, whereas the $C_{16:0}$ and $C_{18:0}$ had microbial origins.

3.3.2 Crick

3.3.2.1 Total lipid extracts

(i) Potsherds

The total lipid content of the potsherds excavated from Crick, shown in the Table 3.7, ranged from 0.7 to 1861 μg g^{-1} lipid. It was decided to focus subsequent investigations on the potsherds containing >15 μg g^{-1} lipid.

Table 3.8: Lipid content of the potsherds excavated at Crick.

Sample	Total lipid content / μg g^{-1}
Crick-A	5.4
Crick-B	538.0
Crick-C	1.9
Crick-D1	11.8
Crick-D2	492.2
Crick-D3	18.4
Crick-D4	222.3
Crick-E	193.1
Crick-F	291.1
Crick-G	1.8
Crick-H	1.1
Crick-I	3.4
Crick-J1	442.7
Crick-J2	0.7
Crick-K	58.8
Crick-L	1861.3

Table 3.6: $\delta^{13}C$ values of individual fatty acids from the burial soils from Eton.

	$\delta^{13}C$ (‰)												
	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	$C_{23:0}$	$C_{24:0}$	$C_{25:0}$	$C_{26:0}$	$C_{27:0}$	$C_{28:0}$	$C_{29:0}$	$C_{30:0}$	$C_{31:0}$
Sample													
Sed-2	-26.7±0.2	-24.6±0.3	-29.8±0.3	-32.0±0.5	-32.9±0.3	-31.9±0.2	-36.0±0.3	-33.1±0.3	-32.3±0.5	-33.6±0.1	-33.8±0.4	-33.9±0.1	-35.1±0.3
Sed-3	-22.3±0.2	-19.1±0.3	-28.7±0.5	-31.6±0.6		-31.9±0.2		-33.4±0.5		-33.9±0.3		-34.2±0.2	
Sed-8	-26.8±0.1	-25.1±0.2	-31.4±0.4	-32.3±0.1	-32.7±0.3	-32.6±0.3	-35.0±0.3	-33.5±0.3	-34.0±0.2	-34.0±0.3	-33.9±0.4	-34.3±0.3	
Sed-15	-26.7±0.4	-24.8±0.1		-33.1±0.1		-32.5±0.3		-33.0±0.4		-34.2±0.2		-33.6±0.1	

The HTGC traces of the residues from the potsherds Crick-B, D2, D4, F, K and L were very similar. The HTGC trace of the residues from Crick-D4 is shown in Figure 3.12a as an example. The preservation of these residues was very good, as they contained significant quantities of triacylglycerols. Partial hydrolysis had taken place, resulting in the formation of mono- and diacylglycerols, and free fatty acids. The free fatty acid distribution was dominated by $C_{18:0}$ and $C_{16:0}$. The odd-numbered $C_{15:0}$ and $C_{17:0}$, and the medium-chain $C_{12:0}$ and $C_{14:0}$ fatty acids were minor constituents of the residues. The triacylglycerol distributions, shown in Figure 3.13 (and, to a certain extent, the mono- and diacylglycerol distributions) were quite wide, ranging from C_{42} to C_{54} (except for Crick-D2, in which the triacylglycerol distribution ranged only from C_{48} to C_{54}). Such profiles are characteristic of degraded animal fats. The presence of odd-numbered fatty acids has been associated with degraded ruminant fats (Charters, 1996; Dudd, 1999), whereas the presence of shorter-chain fatty acids, such as $C_{12:0}$ and $C_{14:0}$, and wide acylglycerol distributions, have been identified as being characteristic of degraded dairy fats (Dudd, 1999). These assignments remain to be confirmed by studying the stable carbon isotope ratios of the $C_{16:0}$ and $C_{18:0}$ fatty acids (Section 3.3.2.2).

Figure 3.12b shows the partial HTGC trace of the residue from Crick-J1. It is dominated by $C_{18:0}$, with $C_{14:0}$ and $C_{16:0}$ present at much lower abundance. Free fatty acids containing up to 26 carbon atoms, with a predominance of the even-numbered compounds, likely originating from plant material (Naraoka and Ishiwatari, 2000) were present in low abundance in the residue. Moderate amount of triacylglycerols, in decreasing abundance from C_{48} to C_{54}, were detected, but there were no mono- and diacylglycerols in this residue.

Figure 3.12c shows the partial HTGC trace of the residue from the potsherd Crick-D2. It shows the same compounds as in the potsherd Crick-J1, but the triacylglycerol distribution is similar to that observed in the extracts of Crick-B, D2, D4, F, K and L. Both residues shown in Figures 3.12a and 3.12b presented very unusual compositions that have not been reported in the literature. The strong dominance of $C_{18:0}$ over the other fatty acids, the presence of long-chain fatty acids and the unusual triacylglycerol distribution in Crick-J1 makes the identification of these residues difficult. However, they are likely to result from the processing of animal and plant products in the vessels.

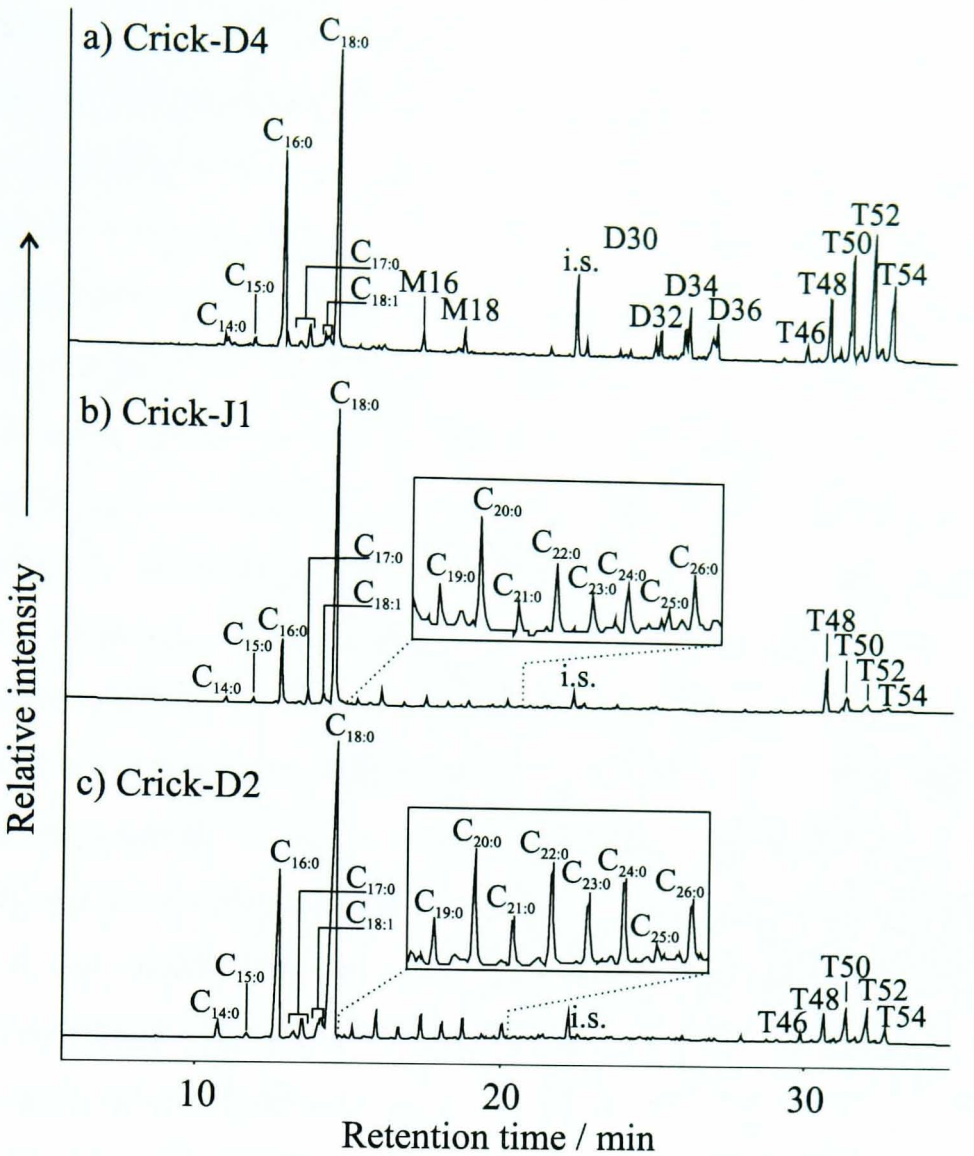


Figure 3.12: Partial HTGC traces of the trimethylsilylated total lipid extracts from the potsherds a) Crick-D4, b) Crick-J1 and c) Crick-D2. Peak identities: $C_{m:n}$ = fatty acid with m acyl carbon atoms and n double-bonds; M16 and M18 = monoacyglycerols containing 16 and 18 acyl carbon atoms; i.s. = internal standard (*n*-tetratriacontane); D30-D36 = diacylglycerols containing 30 to 36 carbon atoms; T46-T54 = triacylglycerols containing 46 to 54 acyl carbon atoms.

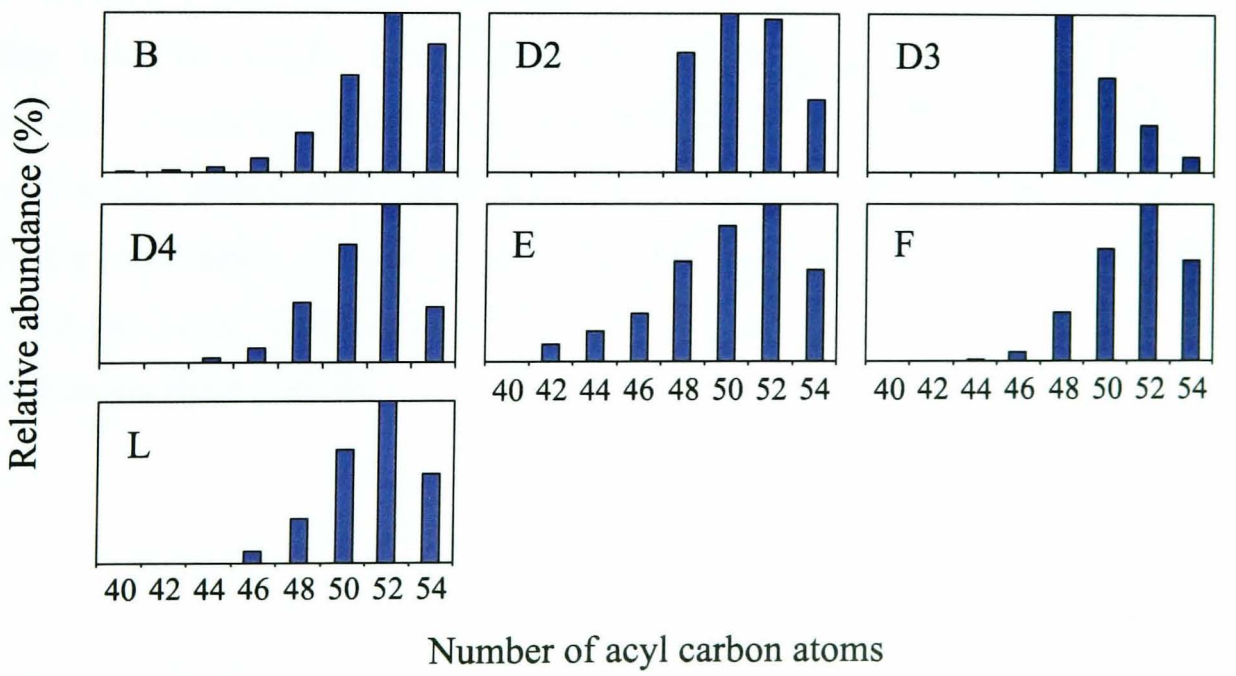


Figure 3.13: Triacylglycerol distributions in the total lipid extracts from the potsherds from Crick.

Figure 3.14 shows the partial HTGC trace of the residue from the potsherd Crick-E. It shows the same components described above in the potsherds Crick-B, D2, D4, F, K and L but also contains long-chain *n*-alcohols, *n*-alkanes, ketones and wax esters. The wax esters contained 40 to 48 carbon atoms, and were identified by HTGC-MS as esters of $C_{16:0}$ and $C_{18:0}$ fatty acids with long chain *n*-alcohols ranging from 22 to 32 carbon atoms. Beeswax contains wax esters consisting exclusively of esters of $C_{16:0}$ with long-chain alcohols (Heron *et al.*, 1994), so the wax esters observed here are likely to have a plant origin. The long-chain *n*-alcohols ranging from 26 to 32 carbon atoms (only compounds with even-numbered carbon atoms were detected) probably arise from hydrolysis of the wax esters. The *n*-alkanes contained between 21 and 31 acyl carbons (only compounds with odd-numbered carbon atoms were detected). Long-chain *n*-alkanes have previously been identified in residues extracted from cooking pots and they are generally accepted to arise from the processing of leafy vegetables (Bland, 1999). Finally, the ketones untriacontan-16-one (C_{31}), tritriacontan-16-one (C_{33}) and pentatriacontan-18-one (C_{35}) were detected in the residue. Evershed and co-workers (1995) demonstrated that these compounds are produced during the heating of fatty acids at high temperature (>300°C) in unglazed ceramic vessels (Evershed *et al.*, 1995; Raven *et al.*, 1997). Overall, the lipid residue from Crick-E could have originated from the simultaneous or consecutive processing of animal (probably dairy) and vegetable commodities.

The preservation in the samples from Crick varied significantly between vessels. Figure 3.15 shows the free fatty acid to triacylglycerol ratio for these samples. It ranged between 0 (for residues containing no free fatty acids) and almost 7 (for residues showing abundant free fatty acids), as triacylglycerol hydrolysis and free fatty acid leaching affected the residues to very different extents during the burial of these samples. This observation was unexpected as these potsherds have been excavated from the same site, and, hence, have been exposed to very similar climatic conditions during burial. It is likely that the burial conditions are not the most important parameters governing the extent of decay during burial.

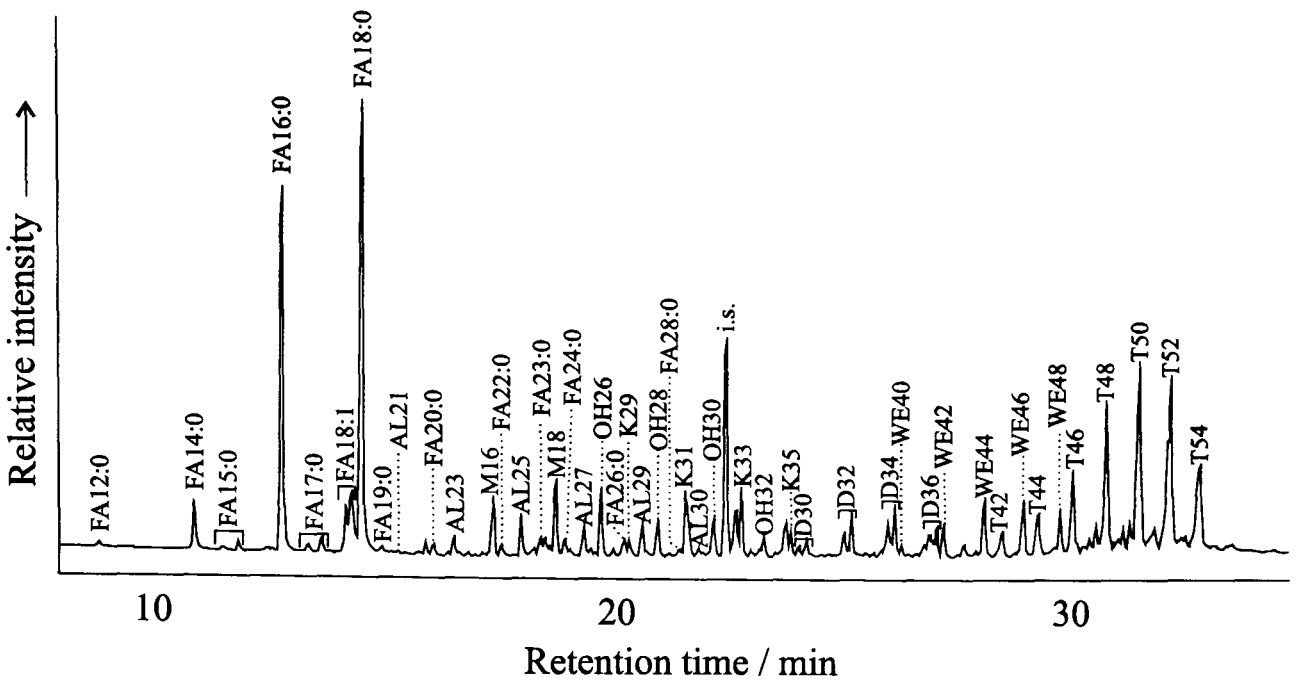


Figure 3.14: Partial HTGC trace of the trimethylsilylated total lipid extract from the potsherd Crick-E. Peak identities: FAM:N = fatty acids with M acyl carbon atoms and N double-bonds; AL21-AL31 = *n*-alkanes containing 21 to 31 carbon atoms; M16 and M18 = monoacyglycerols containing 16 and 18 acyl carbon atoms, respectively; OH26-OH32 = *n*-alcohols containing 26 to 32 carbon atoms; K31-K35 = ketones containing 31 to 35 carbon atoms; i.s. = internal standard (*n*-tetratriacontane); D30-D36 = diacylglycerols containing 30 to 36 acyl carbon atoms; WE40-WE48 = wax esters containing 40 to 48 acyl carbons; T46-T54 = triacylglycerols containing 46 to 54 acyl carbon atoms.

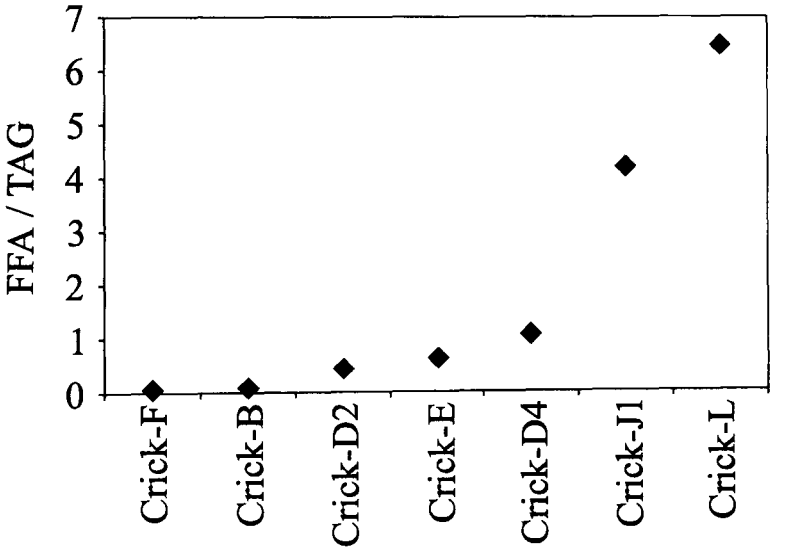


Figure 3.15: Free fatty acid (FFA) to triacylglycerol (TAG) ratios for the potsherds from Crick.

Table 3.8: Physico-chemical properties of the burial soils from Crick.

Sample	Lipid content / $\mu g\ g^{-1}$	PH	CEC / $cmol_c\ kg^{-1}$	Organic carbon content (%)	C/N	C/P	Carbonate content (%)	Clay content (%)	Textural class
A	98.8	6.8	274	0.65	9.2	3.8	0.04	7.1	Sandy silt loam
B	124.4	5.9	202	0.87	7.2	3.3	0.04	3.7	Sandy loam
C	50.6	6.3	336	0.55	9.1	2.3	0.03	4.0	Sandy loam
D	30.3	6.6	532	1.06	9.6	3.0	0.02	4.7	Sandy loam
E	79.0	6.4	412	1.18	6.9	2.7	0.06	4.7	Sandy loam
F	46.0	6.7	526	0.78	9.8	2.8	0.02	5.7	Sandy loam
G	27.6	6.4	535	0.54	4.9	1.6	0.02	6.0	Sandy loam
H	141.9	6.6	137	0.98	8.9	2.2	0.02	4.5	Sandy loam
I	53.8	6.4	140	0.82	6.8	2.1	0.03	3.3	Sandy loam
J	88.0	5.3	228	1.57	9.2	7.1	0.02	4.3	Sandy loam
K	62.5	6.8	329	1.04	6.5	3.9	0.02	5.1	Sandy silt loam
L	48.5	6.2	252	0.88	6.5	4.9	0.04	2.8	Sandy loam

(ii) Burial soils

The physico-chemical properties of the burial soils from Crick are shown in the Table 3.8. All soils were slightly acidic, with a pH ranging between 5.3 and 6.8. The soils were classified as sandy loam and sandy silty loam, with clay contents of 2.8 to 7.1 %. Both the C/P and C/N meet the requirements of soil microorganisms for soil organic matter decomposition (Lallier-Verges *et al.*, 1998). The total lipid content of the burial soils from Crick, shown in Table 3.8, ranged between 30 and 142 μg g^{-1} lipid. HTGC analysis of the total lipid extract from the burial soils revealed the similar distributions of components and Figure 3.16 shows the partial HTGC trace of the residue from Sed-E as an example. It is dominated by fatty acids, ranging from $C_{16:0}$ to $C_{34:0}$, and also contained series of *n*-alcohols and fatty acids. As archaeological interpretations rely increasingly on the study of the stable isotope ratio of fatty acids, it was decided to focus further investigation on the fatty acid fraction of the burial sediments.

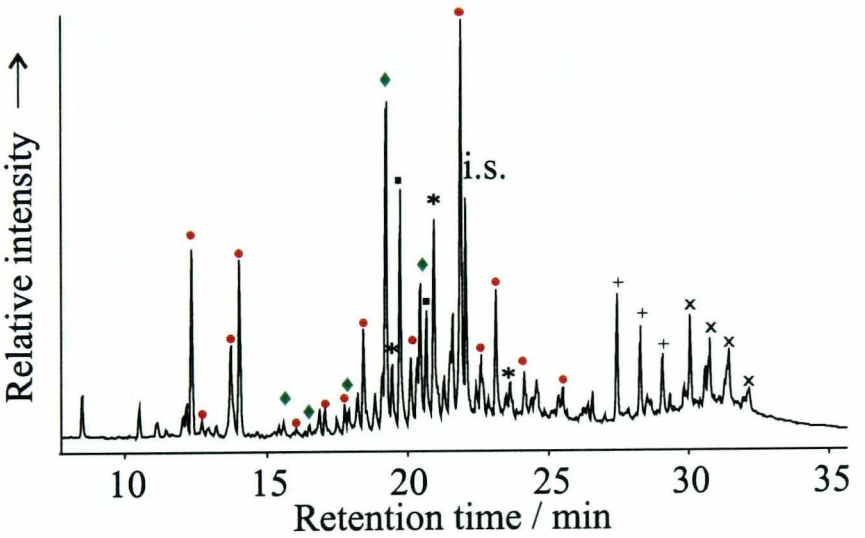


Figure 3.16: Partial HTGC trace of the trimethylsilylated total lipid extract from the burial soil Sed-E. Peak identities: ´ = fatty acids; ■ = *n*-alkanes; ◆ = fatty alcohol; * = ω-hydroxy fatty acids; + = wax esters; x = triacylglycerols.

3.3.2.2 Fatty acid fractions

(i) Potsherds

Table 3.10 shows the fatty acid composition of the lipid extracts of the potsherds from Crick. All residues were dominated by $C_{18:0}$ (20.5 – 70.1%) and $C_{16:0}$ (21.9 – 44.7%). The medium-chain fatty acids $C_{12:0}$ and $C_{14:0}$ were identified in low abundance (up to 8.5%). The longer-chain fatty acids, containing up to 24 carbon atoms were present in the samples Crick-E and Crick-D2, samples for which these compounds had been observed in the total lipid extract. The unsaturated fatty acid $C_{18:1}$ accounted for between 0 and 12.5% of the fatty acids; DMDS derivatives were prepared in order to determine the isomers present.

Table 3.9: Fatty acid distributions in the total lipid extract from the potsherds from Crick.

	Relative abundance (%)							
Sample	Crick-B	Crick-D2	Crick-D4	Crick-E	Crick-F	Crick-J1	Crick-K	Crick-L
Fatty acid								
$C_{12:0}$	0	0	0	0.3	0	0	0	0
$C_{14:0}$	3.5	1.2	3.6	6.7	8.5	2.4	3.1	1.1
$C_{15:0}$	1.6	0.8	2.0	1.9	1.2	1.8	1.2	0.4
$C_{16:1}$	0	0	0	2.3	0.2	0	0	0.4
$C_{16:0}$	28.6	29.4	38.7	44.7	36.5	25.3	37.3	21.9
$C_{17:0}$	5.5	5.4	6.2	3.5	5.2	4.9	7.6	3.1
$C_{18:1}$	12.5	0	4.6	4.8	6.5	1.9	0	3.0
$C_{18:0}$	48.3	48.4	44.9	20.5	41.8	63.7	50.9	70.1
$C_{19:0}$	0	0.6	0	0	0	0	0	0
$C_{20:0}$	0	1.5	0	1.2	0	0	0	0
$C_{21:0}$	0	0.9	0	0.5	0	0	0	0
$C_{22:0}$	0	1.5	0	8.2	0	0	0	0
$C_{23:0}$	0	10.4	0	1.4	0	0	0	0
$C_{24:0}$	0	0	0	4.0	0	0	0	0

The relative abundance of the $C_{18:1}$ isomers in the potsherds Crick-B, D4, E and F are shown in Figure 3.17. The residues all contained high abundances of *cis*-$C_{18:1}\Delta^8$ and *trans*-$C_{18:1}\Delta^{13}$, and the 7 isomers of $C_{18:1}$ with the double-bond in a *trans* configuration were present in low abundance in all samples. The $C_{18:1}$ isomers distribution in all the samples were quite similar to that of cows milk fat (see Section 5.2.1.3), except that the relative abundance of oleic acid (*cis*-$C_{18:1}\Delta^9$) was much lower than in cows milk fat. Preferential depletion of fatty acids with double-bonds possessing the *cis* configuration has been previously observed in degraded animal fats absorbed in unglazed ceramics (Dudd, 1999), and the possible explanations for this are discussed in Section 3.3.1.2.

Figure 3.18 shows the $\delta^{13}C$ of the fatty acid $C_{16:0}$ and $C_{18:0}$ for the potsherds from Crick. All samples are clustered around $\delta^{13}C_{16:0}$ = -27 to –29‰ and $\delta^{13}C_{18:0}$ = -29 to –33‰. The values for the samples Crick-D2, E and J1, whose total lipid extracts showed significant input of plant material, were similar to those of the other samples, whose total lipid extracts were characteristic of degraded animal fats. All the values plotted along the mixing curve between ruminant adipose and/or dairy fats and pig fat, which would be consistent with the interpretation based on the total lipid extracts.

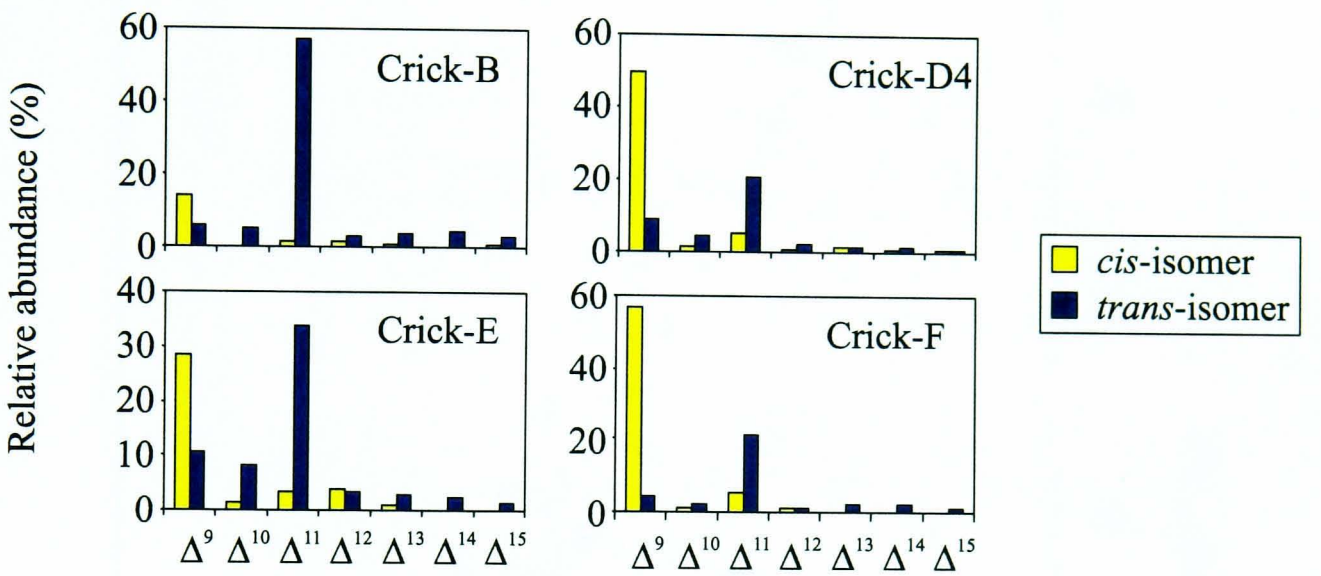


Figure 3.17: Distribution of the $C_{18:1}$ isomers in the potsherds from Crick.

(ii) Burial soils

As was observed in Eton (Section 3.3.1.2), the fatty acid distributions for the burial soils was significantly different to that in the ceramic residues, as shown in Figure 3.19. All soil samples contained saturated fatty acids with 16 to 35 carbon atoms. Samples Sed-B, Sed-D and Sed-F contained significant abundances of the longer-chain fatty acids, whereas Sed-E, Sed-J and Sed-L were largely dominated by $C_{16:0}$ and $C_{18:0}$. All residues were dominated by the even-carbon-number fatty acids (the CPIs ranged between 2.9 for Sed-B and 5.0 for Sed-D).

Table 3.11 shows the $\delta^{13}C$ values of the fatty acids from the soils from Crick. The values for the $C_{16:0}$ and $C_{18:0}$ fatty acids ranged between –27.8 and –29.0‰, and –27.9 and –29.9‰, respectively. The longer chain fatty acids were all more depleted, with values ranging between –31.7 and –36.0‰. The difference between the values for these 2

groups of fatty acids arises from the different origin of these compounds as $C_{16:0}$ and $C_{18:0}$ are likely of microbial origin, whereas the longer-chain fatty acids originate from plants (Lichtfouse *et al.*, 1995; see Section 3.3.1.2).

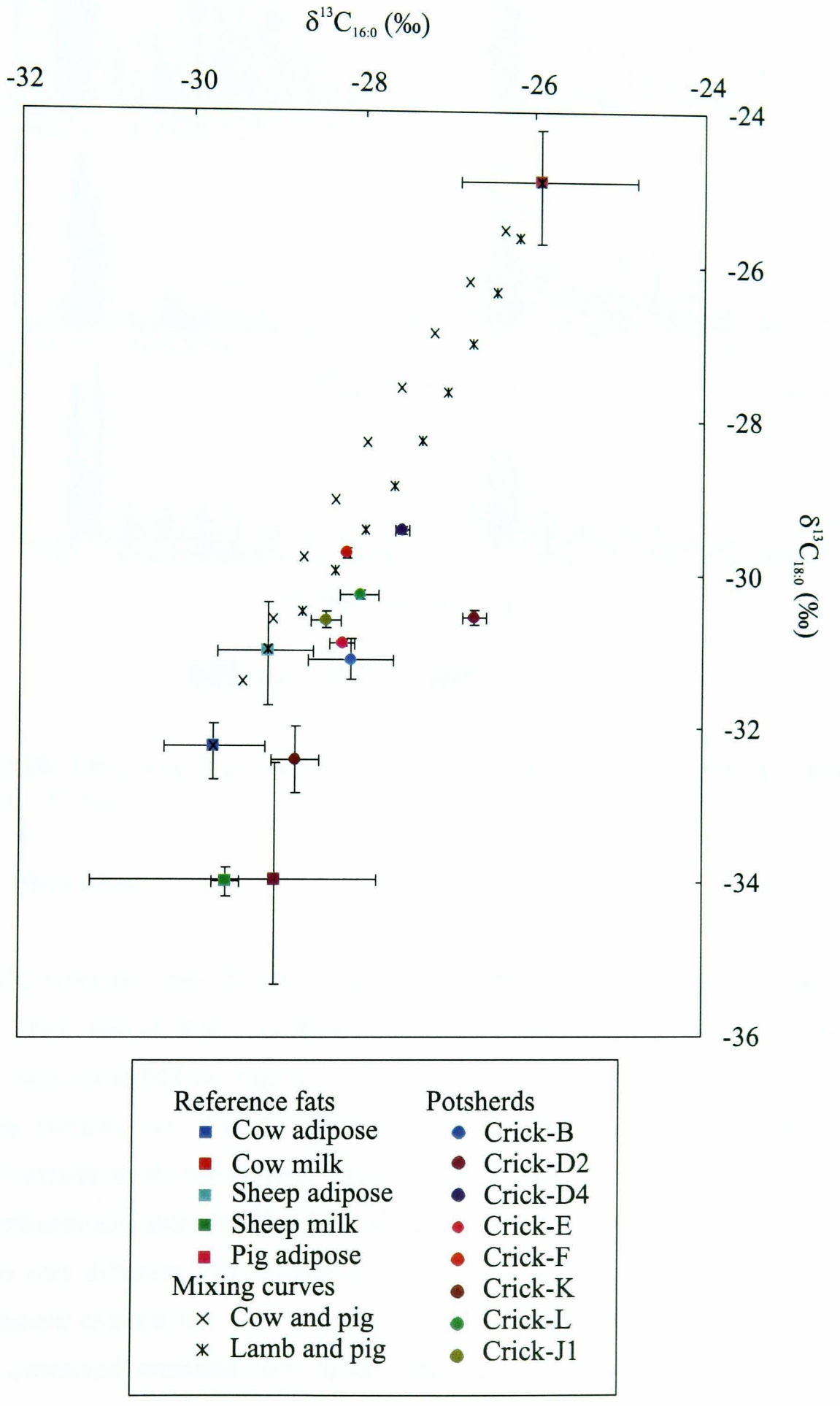


Figure 3.18: $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ from the potsherds from Crick, compared to reference animal fats (Dudd, 1999).

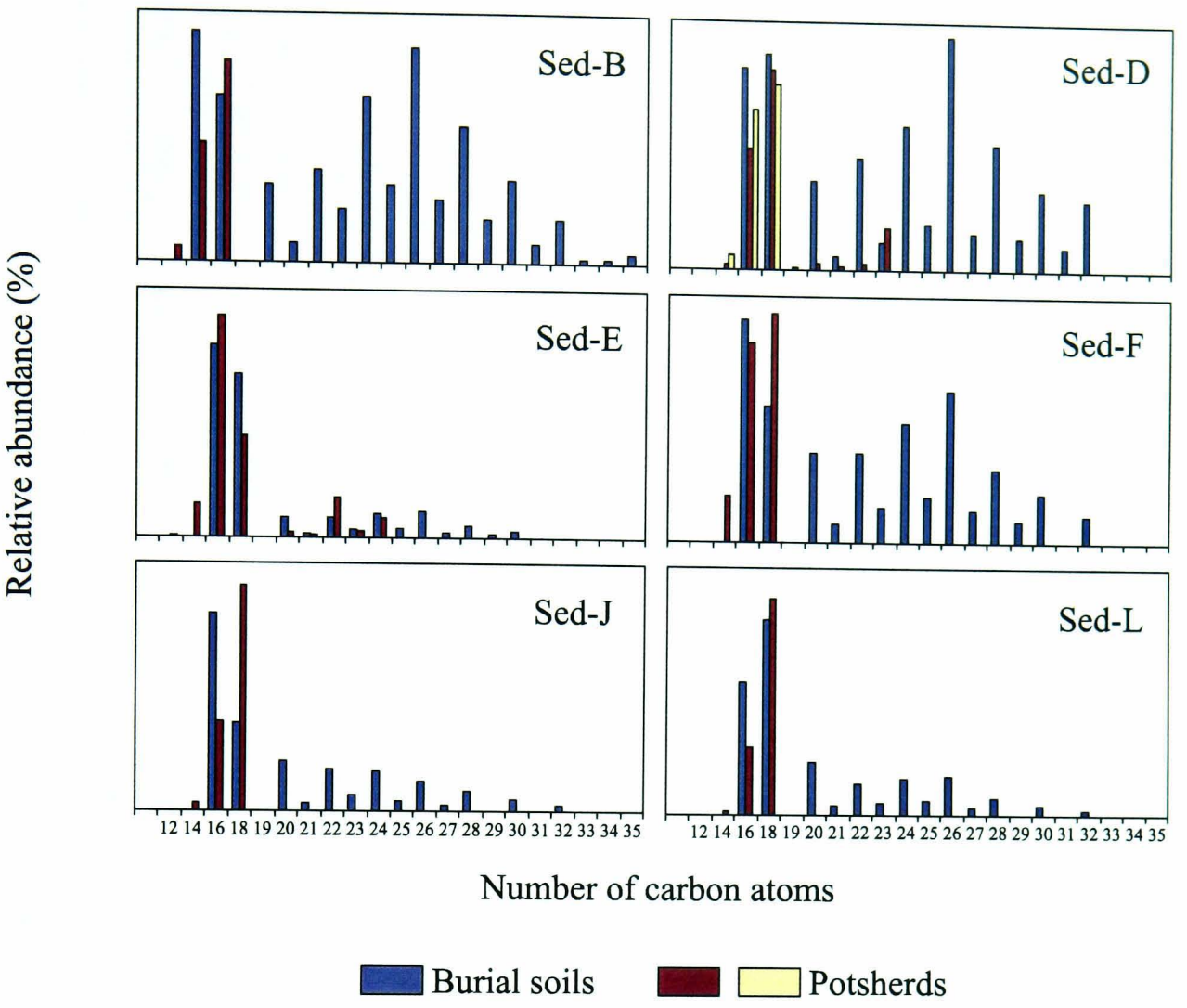


Figure 3.19: Fatty acid distributions in the total lipid extracts from the potsherds and burial soils, Crick.

3.4 Discussion

The HTGC profiles of the solvent-extractable fractions from the potsherds extracted from Eton or Crick and of their immediate burial soils were very different. All potsherds residues were identified as degraded animal fats based on the distribution of their acyl lipids and extracts from some potsherds showed contribution from a vegetable source. The soil extracts were very complex and consisted mainly of series of long-chain fatty acids, *n*-alkanes and alcohols. The fatty acid distributions in the potsherd and soil extract were also very different. The medium-chain fatty acid $C_{12:0}$ and $C_{14:0}$ were identified in some potsherd extracts but were not present in any of the soil extracts. Conversely, soil samples contained saturated fatty acids with up to 35 carbon atoms that were not identified in the majority of the potsherds extracts.

Table 3.10: $\delta^{13}C$ values for individual fatty acids in the burial soils from Crick.

	$\delta^{13}C$ (‰)													
	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{21:0}$	$C_{22:0}$	$C_{23:0}$	$C_{24:0}$	$C_{25:0}$	$C_{26:0}$	$C_{27:0}$	$C_{28:0}$	$C_{29:0}$	$C_{30:0}$	$C_{31:0}$
Samples														
Sed-B	-28.2 ± 0.1	-27.9 ± 0.2	-32.7 ± 0.2	-32.1 ± 0.1	-32.7 ± 0.2	-32.8 ± 0.1	-32.8 ± 0.2	-35.3 ± 0.1	-33.8 ± 0.1	-34.0 ± 0.1	-34.6 ± 0.1	-34.6 ± 0.3	-34.8 ± 0.1	-36.0 ± 0.1
Sed-D	-27.8 ± 0.2	-28.4 ± 0.2	-31.7 ± 0.8	-	-32.7 ± 0.2	-32.6 ± 0.2	-33.1 ± 0.3	-35.4 ± 0.5	-33.8 ± 0.1	-33.8 ± 0.2	-34.5 ± 0.1	-34.0 ± 0.3	-34.4 ± 0.1	-35.5 ± 0.3
Sed-E	-29.0 ± 0.1	-29.9 ± 0.1	-32.2 ± 0.6	-	-33.0 ± 0.1	-32.6 ± 0.3	-32.8 ± 0.1	-35.5 ± 0.2	-33.8 ± 0.1	-33.9 ± 0.1	-34.4 ± 0.2	-34.3 ± 0.2	-34.2 ± 0.2	-35.6 ± 0.2
Sed-F	-28.5 ± 0.2	-28.7 ± 0.2	-31.7 ± 0.2	-32.2 ± 0.5	-32.4 ± 0.2	-32.4 ± 0.2	-32.6 ± 0.1	-34.9 ± 0.2	-33.5 ± 0.1	-33.7 ± 0.1	-34.1 ± 0.3	-34.0 ± 0.2	-34.3 ± 0.2	-34.1 ± 0.1
Sed-J	-28.4 ± 0.1	-28.8 ± 0.1	-32.6 ± 0.1	-31.8 ± 0.3	-32.5 ± 0.2	-32.6 ± 0.3	-32.8 ± 0.1	-35.5 ± 0.2	-33.7 ± 0.1	-33.6 ± 0.5	-34.6 ± 0.3	-34.2 ± 0.5	-34.7 ± 0.2	-34.4 ± 0.4
Sed-L	-28.4 ± 0.2	-29.2 ± 0.2	-31.7 ± 0.3	-32.6 ± 0.3	-32.7 ± 0.2	-32.8 ± 0.1	-32.9 ± 0.3	-34.4 ± 0.2	-33.1 ± 0.2	-34.0 ± 0.3	-34.4 ± 0.4	-35.4 ± 0.2	-32.9 ± 0.1	-36.0 ± 0.2

Furthermore, when long-chain alcohols were identified in ceramic extracts, there was other evidence in the TLE to suggest that these compounds had a vegetable origin, such as the presence of wax esters, alcohols or alkanes, and had not been absorbed from the immediate burial sediments during burial. Finally the ratios $C_{16:0}/C_{18:0}$, traditionally used for the identification of degraded archaeological residues, were relatively different for potsherds and soil extracts, and more importantly, there was no correlation between the values for potsherds and soil extracts (Table 3.11).

Table 3.12: $C_{16:0}/C_{18:0}$ ratios in the potsherd and soil solvent-extractable fractions from Eton and Crick.

	$C_{16:0}/C_{18:0}$	
	Potsherds	Burial soils
Eton		
2/1	2.8	0.9
2/2	1.7	0.9
3	1.3	0.7
8/1	1.8	1.0
8/2	1.5	1.0
15	1.8	0.9
Crick		
B	0.6	1.4
D2	0.6	0.9
D4	0.9	0.9
E	2.2	1.2
F	0.9	1.6
J1	0.4	2.3
L	0.3	0.7

Figures 3.20 and 3.21 show the stable carbon isotope ratios of $C_{16:0}$ and $C_{18:0}$ in the potsherd and soil extracts from Eton and Crick, respectively. In the samples from Eton, the $C_{16:0}$ and $C_{18:0}$ fatty acids from the potsherd extracts were always more depleted than the fatty acids from the soil extracts, and there was a significant difference between the values for the potsherds and soil extracts. In the samples from Crick, the $C_{16:0}$ fatty acid was more depleted in the soil extracts than in the potsherd extracts, whereas the reverse situation was observed for the $C_{18:0}$ fatty acid. This is a very significant finding, as $\delta^{13}C$ values for individual fatty acids are extensively used in the identification of degraded archaeological residues, especially degraded dairy fats (Dudd, 1999). The significant differences observed here between the values for the burial soils and the ceramic extracts confirms the robustness of the $\delta^{13}C$ values during burial and bring more confidence to the identification of archaeological residues based on stable carbon isotope studies.

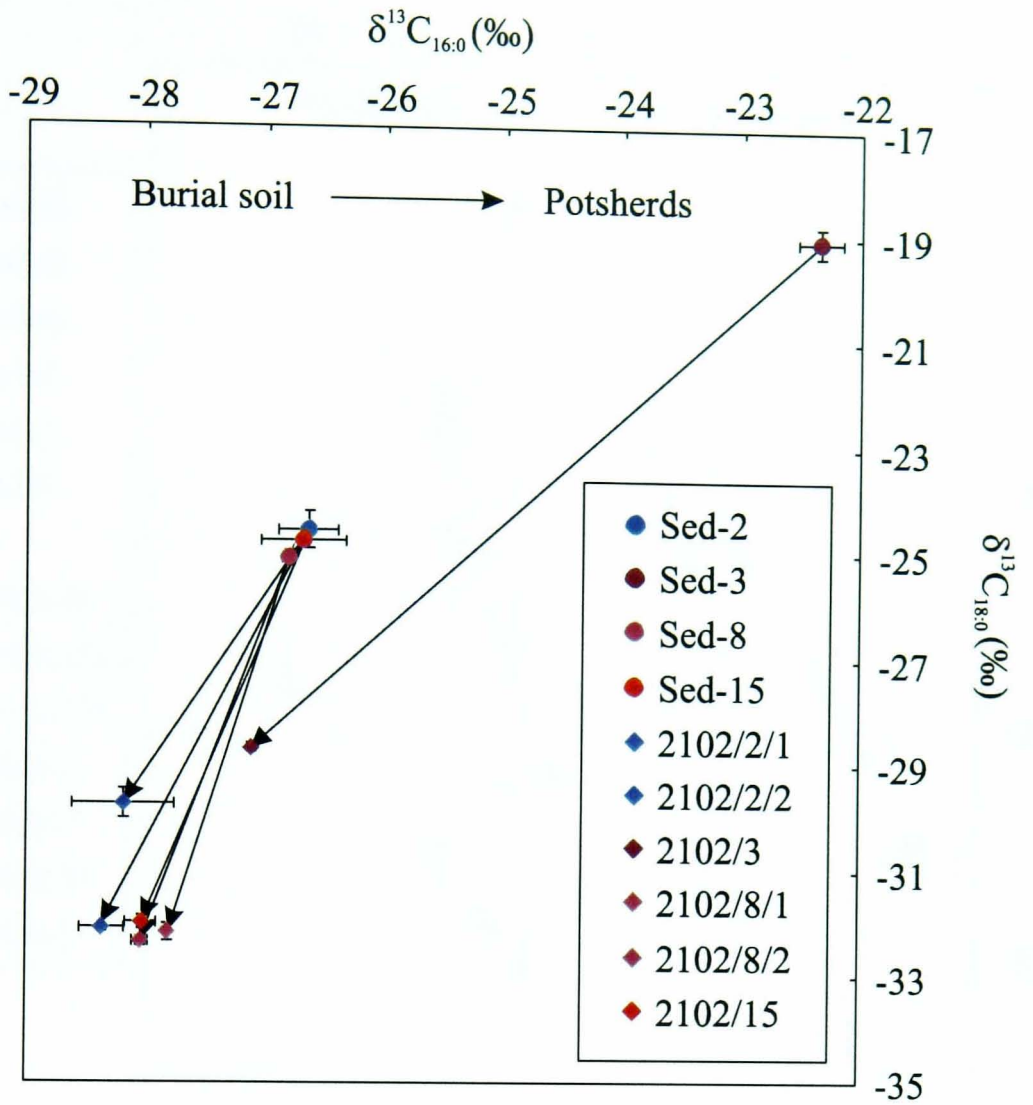


Figure 3.20: Comparison between the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in the solvent-extractable fractions from the potsherds and soils from Eton.

3.5 Conclusions

This chapter aimed at assessing the effect of lipid absorption from burial soils on the composition of ceramic extracts, and subsequently, on the identification of residues from archaeological ceramics. Several important compositional criteria commonly used in the identification of archaeological residues, including fatty acid distribution and $\delta^{13}C$ values for individual fatty acids, were considered. The chemical compositions of the ceramic extracts were significantly different to that of the embedding soils, which inferred that the lipids recovered in the ceramic extracts were likely to be endogenous. This was confirmed by the significant difference between the $\delta^{13}C$ values between individual fatty acids in potsherds and in soils. These findings are very important as they enhance the value of the identification of degraded archaeological residues based on the study of the stable carbon isotope values of some of their individual components, especially fatty acids.

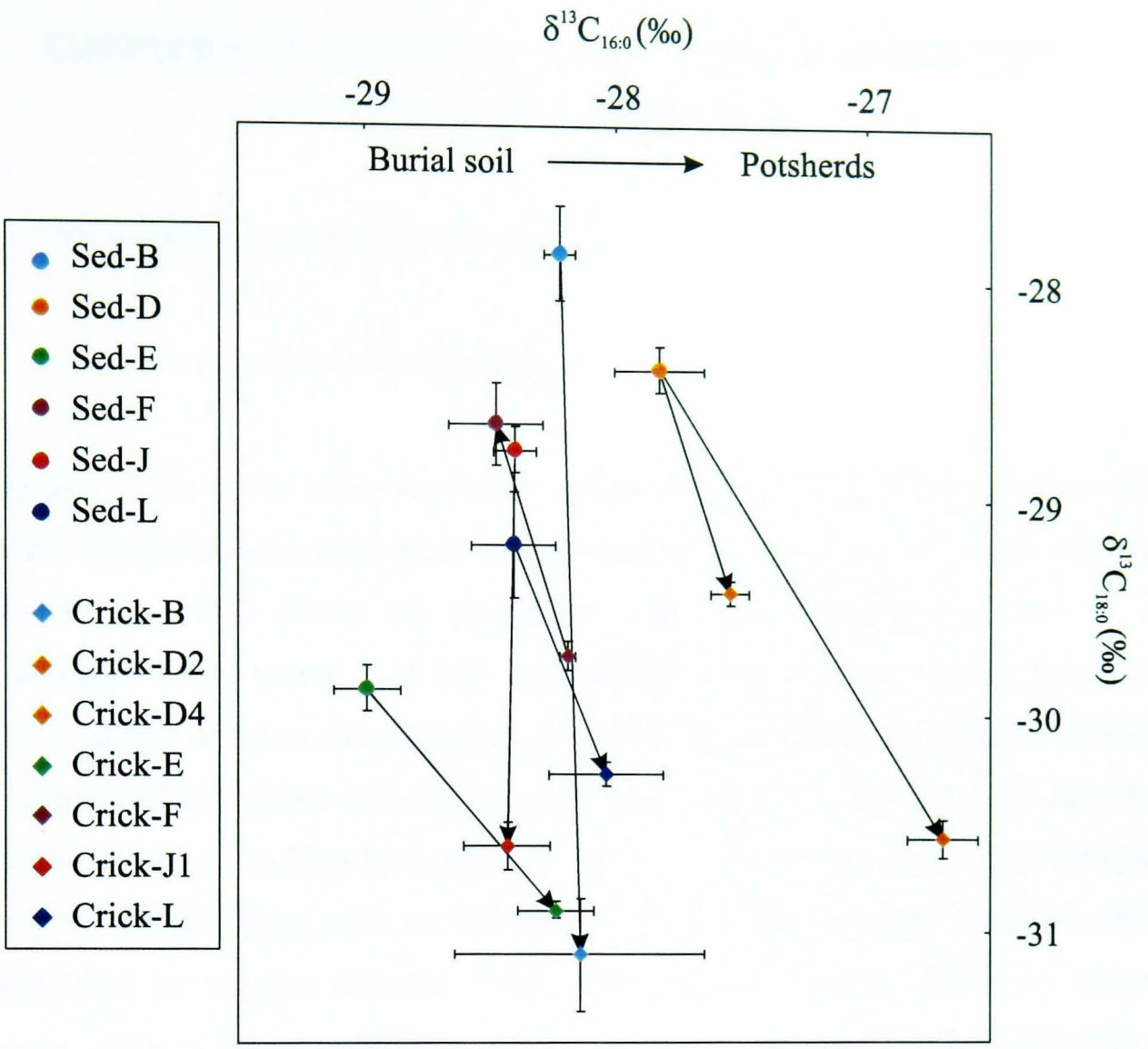


Figure 3.21: Comparison between the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in the solvent-extractable fractions from the potsherds and soils from Crick.

CHAPTER 4: EXPERIMENTAL DECAY OF OLIVE OIL UNDER LABORATORY CONDITIONS

4.1 Introduction and aims of the chapter

4.1.1 Importance of olive oil in Antiquity

The domestication of the olive tree (*Olea europaea* L.) has been dated to around 6000 BC and is thought to have taken place independently both in modern day Syria and Crete (Galili *et al.*, 1997). Olive oil production was soon extended to most of the Mediterranean basin, where it is still concentrated today (Greece, Spain, Italy). The earliest evidence of olive oil production, including stone basins, mortars and grinding tools, associated with intact and crushed olive stones and olive pulp, has been identified in modern day Israel and has been dated to 6000 BC (Galili *et al.*, 1997). In Antiquity, and until the Middle-Ages, olive oil was widely exploited as a foodstuff (Renfrew, 1972), an illuminant in temples (Meeks, 1993) and churches (Grieco, 1993), an unguent (Renfrew, 1972), a lubricant (Fenton, 1988), and a base for pharmaceutical preparations such as rose oil, violet oil and bay oil (Grieco, 1993). Olive oil also found applications in industrial processes such as soap making and cloth processing (Grieco, 1993). Olive oil was extensively traded in Antiquity and was considered as a luxurious article (Meeks, 1993) in Mycenaean Greece. In Roman times, olive oil was stored and traded exclusively in amphora of the type "Dressel 20" (Sealey and Tyers, 1989).

4.1.2 Identification of olive oil in archaeological residues

4.1.2.1 Chemical properties of olive oil

The chemical composition of olive oil has been extensively studied and is well characterised. Intact olive oil consists mainly of triacylglycerols (93%), ranging from C_{48} to C_{54}, with diacylglycerols present only in minor quantity (6%). Free fatty acids account for less then 0.1% (Gunstone *et al.*, 1986). Table 4.1 shows the relative abundance of the different fatty acids released by saponification of olive oil. The main fatty acid in olive oil is oleic acid (55 – 83%) and the majority of the triacylglycerols in olive oil contains at

least 1 double-bond (Gallina Toschi *et al.*, 1993, Gunstone *et al.*, 1986), which makes olive oil very susceptible to processes affecting double-bonds, such as radical oxidation. Minor components have been detected in olive oil, including alkanes, *n*-alcohols (Lanuzza *et al.*, 1996) and sterols of which β-sitosterol is the most abundant (1310 mg kg^{-1} olive oil; Gunstone *et al.*, 1986).

Table 4.1: Relative abundance of the major fatty acids found in olive oil (Quigley, 1992; Gallina Toschi *et al.*, 1993; Gunstone *et al.*, 1986).

Fatty acid	Relative abundance (%)
$C_{16:0}$	7 –20
$C_{18:0}$	0.5 – 4
$C_{16:1}$	0.3 – 3.5
cis-$C_{18:1}\Delta^9$	55 – 83
$C_{18:2}$	3.5 – 20

The stable carbon isotope ratios of individual fatty acids in olive oil have been determined (Royer *et al.*, 1998; Spangenberg *et al.*, 1998) and are shown in Table 4.2.

Table 4.2: Range of the stable carbon isotope ratios of bulk olive oil and of individual fatty acids found in olive oil (Woodbury, 1998, Royer *et al.*, 1998; Spangenberg *et al.*, 1998).

Fatty acid	$\delta^{13}C$ (‰)
$C_{16:0}$	-32.6 to -27.4
$C_{18:1}$	-33.9 to -27.2
$C_{18:2}$	-30.2 to -27.5
Bulk oil	-30.2 to -27.7

4.1.2.2 Identification of olive oil in the archaeological record

Olive oil has seldom if ever been identified with confidence in the archaeological record, probably because of its high degree of unsaturation makes it very susceptible to oxidation during vessel use and burial. Investigation of an Roman amphora filled with olive fruits preserved in liquid was conducted by Sealey and Tyers (1989) but analysis of the organic residue absorbed in the wall of the vessel only enabled the identification of triacylglycerols and sugars. Comparison with ancient carbonised olive fruit enabled Shimoyama and coworkers (1995) to identify organic residues extracted from a series of

Syrian jars and lamps (900-720 BC) as degraded olive oil. They based their conclusions on the relative abundances of the fatty acids $C_{16:0}$ and $C_{18:0}$, but also $C_{18:1}$ in some cases, and on the presence of degradation products including α,ω-dicarboxylic acids and short-chain fatty acids dominated by nonanoic acid. Likewise, by comparing the relative abundance of the $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids, Condamin and coworkers (1976, 1978) and Passi and coworkers (1981) extracted residues of composition consistent with degraded olive oil from an assemblage of Roman amphora and 1500-year-old amphorae and oil lamps, respectively. The exceptional preservation of $C_{18:1}$ was observed in some cases.

4.1.3 Aims of the chapter

Olive oil is suitable for the study of a range of degradation processes as it is susceptible to triacylglycerol hydrolysis and also to radical oxidation because of the high degree of unsaturation. Furthermore, olive oil does not contain any compounds deriving from microorganisms, in contrast to milk, for example, which contains branched and straight-chain odd-numbered fatty acids deriving from the action of bacteria in the rumen (Gunstone *et al.*, 1986). The aims of this chapter are:

(i) To submit olive oil absorbed in unglazed potsherds to experimental decay under a range of laboratory conditions chosen to promote a range of chemical and enzymatic reactions.

(ii) To investigate the decay of the unsaturated components of olive oil under oxidative conditions.

4.2 Influence of the incubation conditions on the rate and extent of decay

4.2.1 Free fatty acid and triacylglycerol relative abundances

Figure 4.1 shows the changes in the triacylglycerol and free fatty acid relative abundance during experiments 1 to 10 (incubation conditions are described in Section 2.3.1). In all experiments, the triacylglycerol relative abundance decreased with time and a concomitant increase of the free fatty acid relative abundance was observed. It was

possible to distinguish between 2 sets of experimental conditions, based on the patterns of change of the relative abundance of the triacylglycerols and free fatty acids.

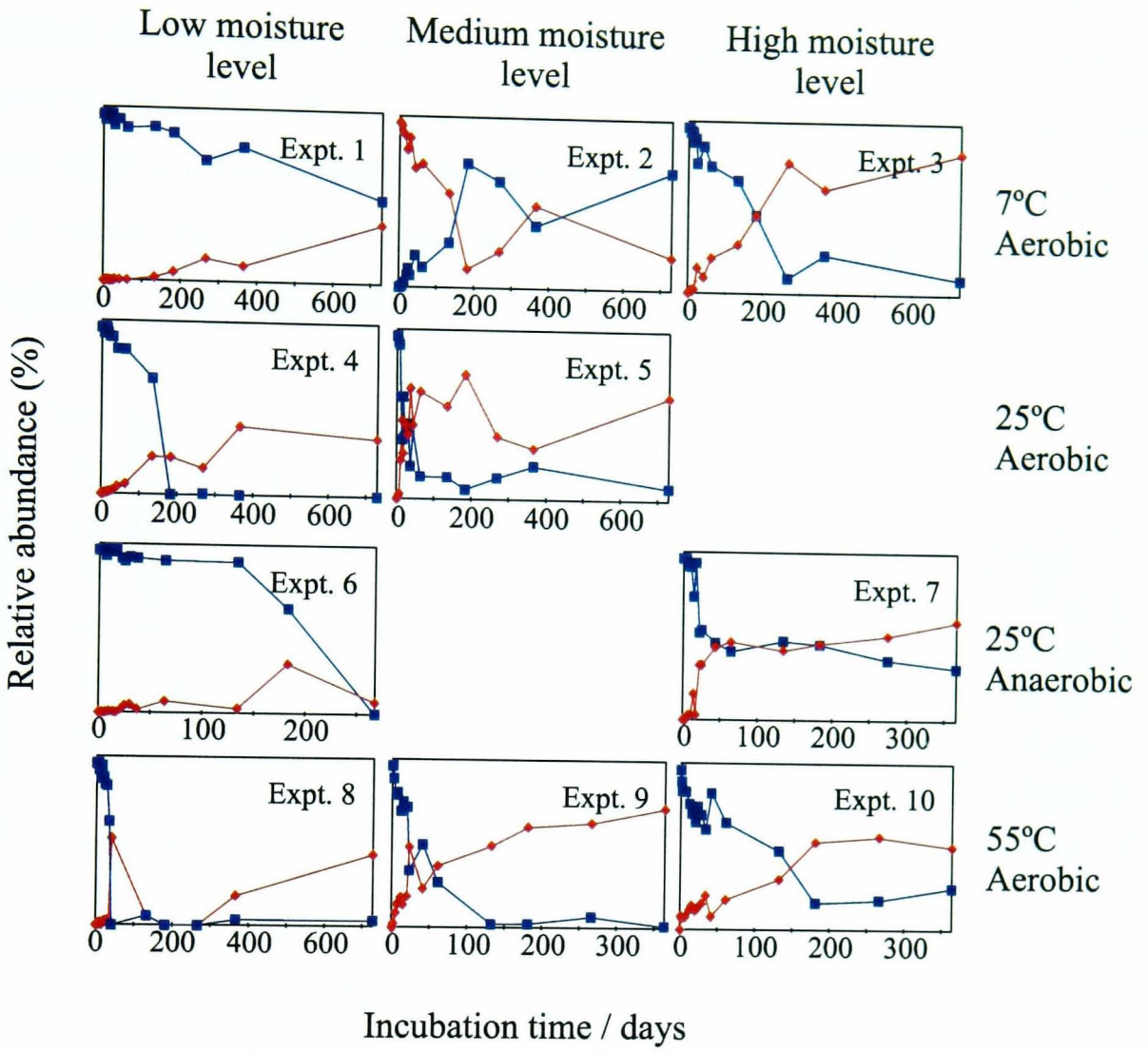


Figure 4.1: Changes in the relative abundance of the triacylglycerols (■) and free fatty acids (◆) during the experimental decay of olive oil under laboratory conditions (Experiments 1-10).

(i) "Hydrolytic" conditions

During experiments 1, 2, 3, 5, 7, 9 and 10, in the first phase of decay which duration is given in Table 4.3, the triacylglycerol relative abundance decreased regularly with time, with the increase of the free fatty acid relative abundance almost exactly mirroring the decrease in triacylglycerol relative abundance. This was especially the case during experiment 1, where the triacylglycerol and free fatty acid relative abundance went from 96% and 0% respectively, at day 0, to 49% and 35%, respectively, after 2 years of incubation. Such behaviour of the free fatty acid and triacylglycerol relative abundances is characteristic of a product-reactant relationship, and is indicative that the free fatty

acids were produced from the triacylglycerols, and that triacylglycerol degradation almost exclusively produced free fatty acids, which is consistent with the mechanism of triacylglycerol hydrolysis. During the first phase of experiments 2, 3, 5, 7, 9 and 10, the evolution of the triacylglycerol relative abundance was almost linear with respect to time, as shown in Figure 4.2, and it was possible to determine best-fit lines with the parameters given in Table 4.3. The triacylglycerol half-life in each experiment was determined as the time at which the triacylglycerol relative abundance was equal to half the initial triacylglycerol relative abundance.

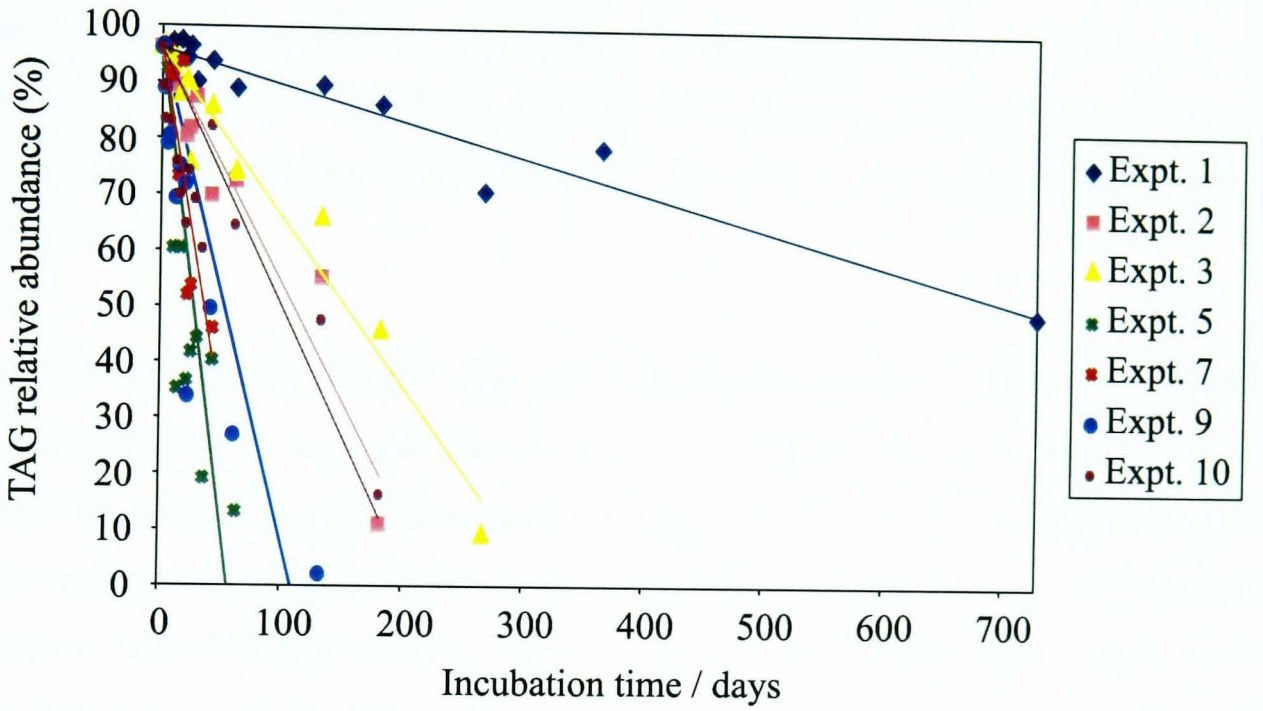


Figure 4.2: Changes in the relative abundance of the triacylglycerols during the laboratory decay of olive oil under "hydrolytic" conditions (Experiments 1, 2, 3, 5, 7, 9 and 10).

Table 4.3: Parameters (duration, slope and R^2 associated with the best-fit lines and half-life) characterising the fist phase of the decay during experiments 1, 2, 3, 5, 7, 9 and 10.

Expt.	Incubation temperature / ° C	Moisture level	Aeration conditions	First phase / days	Slope	R^2	Half-life / days
1	7	Low	Aerobic	0 – 729	-0.06	0.94	801
2	7	Medium	Aerobic	0 – 182	-0.42	0.93	114
3	7	High	Aerobic	0 – 267	-0.30	0.96	160
5	25	Medium	Aerobic	0 – 63	-1.68	0.65	29
7	25	High	Anaerobic	0 – 43	-1.28	0.77	38
9	55	Medium	Aerobic	0 – 132	-0.88	0.66	55
10	55	High	Aerobic	0 – 181	-0.46	0.55	104

The conditions employed in Experiment 1 produced the slowest rate of triacylglycerol hydrolysis (half-life 801 days). Addition of a moderate amount of water to the mushroom compost resulted in a substantial increase in the rate of decay, as shown by the half-life in experiment 2 which was reduced to 114 days. Addition of an excessive amount of water did not increase the rate of decay further, but, to the contrary, decreased it, and the half-life in the experiment 3 was 160 days. The same trend was seen in incubations performed at 55° C, as the half-lives in the experiments 9 and 10 (medium and high water content respectively) were 55 and 104 days, respectively. Experiment 5 was the fastest experiment, displaying a short half-life of 29 days. Increasing or decreasing the temperature did not increase the rate of decay, as the half-lives in the experiment 2 and 9 (7 and 55° C) were 114 and 55 days, respectively. Experiment 7 was conducted under anaerobic conditions, and was only marginally slower than experiment 5, with a half-life of 38 days.

During the experiments 2 and 3, after 180 and 260 days respectively, the free fatty acid abundance started to decrease relative to the triacylglycerols, until day 360 in both experiments, probably as the free fatty acids were consumed by micro-organisms present in the mushroom compost more rapidly than they were produced by triacylglycerol hydrolysis. After 360 days of decay, the triacylglycerol started to decrease again, and that of the free fatty acids to increase. During the experiments 5, 7, 9 and 10, both the free fatty acid and triacylglycerol relative abundances both remained constant at the end of the first phase of the experiment indicated in Table 4.3.

(ii) "Oxidative" conditions

During the experiments 4, 6 and 8, the triacylglycerol relative abundance decreased sharply to almost 0, but with no concomitant increase in the free fatty acid relative abundance being observed. During experiments 4 and 6, the triacylglycerol relative abundance decreased rapidly to 0 after only 200 days of incubation, but the free fatty acid relative abundance only ever reached 40%. During the experiment 8, the triacylglycerol relative abundance decreased sharply to 0 after only 60 days of decay. The free fatty acid relative abundance first increased to 50% but then decreased rapidly to almost 0 after 180 days of decay. This indicated that triacylglycerol degradation produced products other

than free fatty acids, and that triacylglycerol hydrolysis only played a minor role under these conditions.

These observations indicate that experiments 1, 2, 3, 5, 7, 9 and 10 were conducted under conditions favourable to triacylglycerol hydrolysis, called "hydrolytic" conditions hereafter, whereas the experimental conditions under which experiments 4, 6 and 8 were conducted promoted another decay process, which will be identified as unsaturated lipid oxidation in Section 4.4, and these conditions will therefore be called "oxidative" thereafter.

4.2.2 Potsherd lipid and organic carbon content

Figure 4.3 shows the changes in the potsherd lipid content during the experiments 1 to 10. In most experiments, the potsherd lipid content varied significantly during the first 200 days of decay, due to the significant difference in the size of the potsherds used in the experiments, as demonstrated in Section 3.2.1.3. It was nevertheless possible to identify a trend in the potsherd lipid content during the latest stage of decay. For example in experiments 1, 2 or 4, 5, 8, 9 and 10 the potsherd lipid content decreased significantly, until only 0.2 (Experiment 5) to 10.5% (Experiment 1) of the original lipid content remained in the potsherd.

Figure 4.4 shows the potsherd organic carbon content before extraction, after extraction, and after alkaline hydrolysis during experiments 1 to 10. In all experiments, a marked decrease in the potsherd organic carbon content was observed, from 1% before incubation to between 0.1 and 0.8% after 2 years of decay (experiment 6 and 3, respectively). A significant amount of carbon remained after solvent-extraction, accounting for 6 to 89 % of the organic carbon content before extraction, presumably either because the extraction procedure was not exhaustive, but more probably because of the alteration of the nature of the interactions between the lipid residue and the ceramic matrix (see Section 4.5.2 for more details). Alkaline hydrolysis of the extracted potsherd released some of the organic compounds remaining in the ceramic matrix after solvent-extraction.

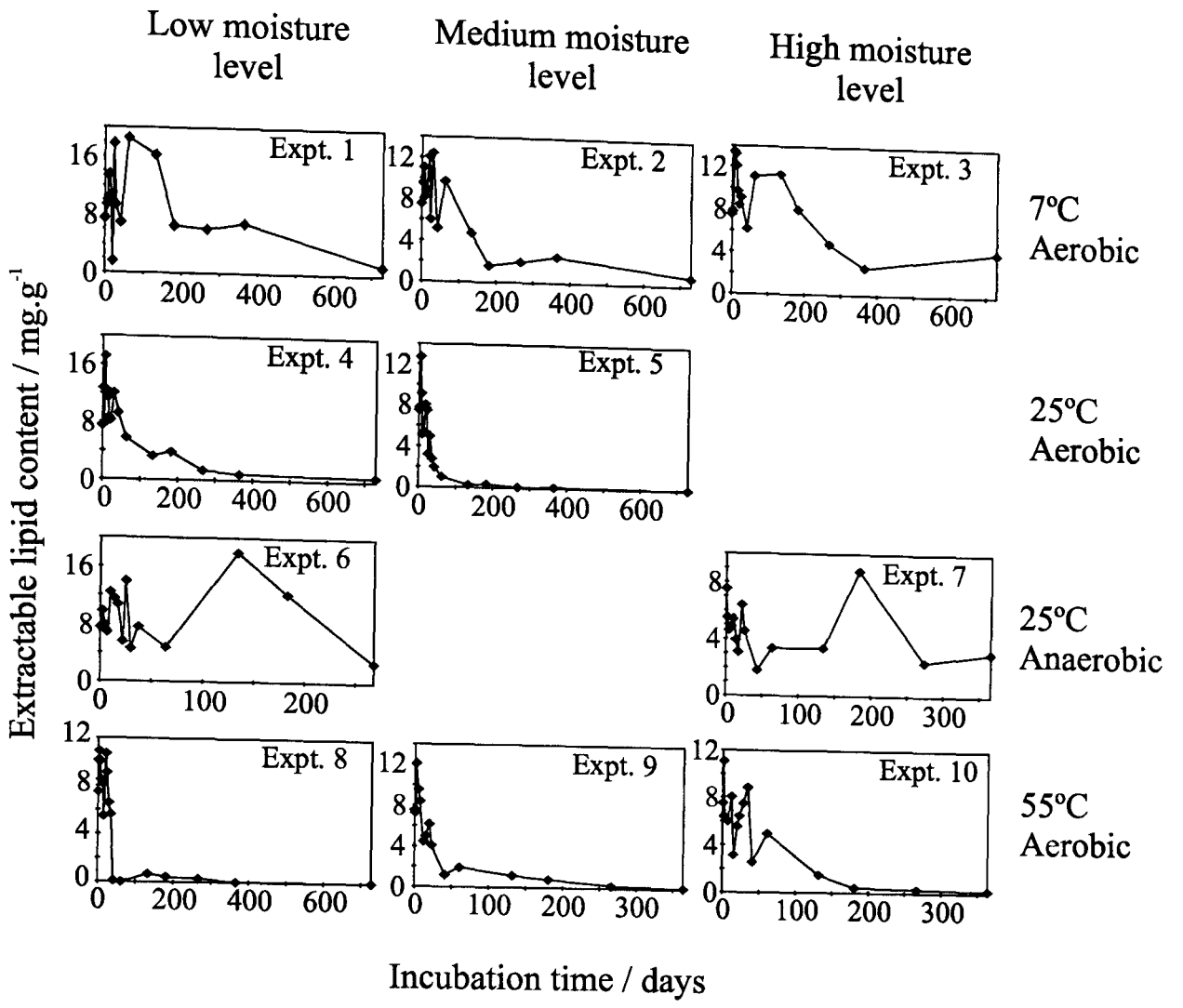


Figure 4.3: Changes in the potsherds lipid content during the decay of olive oil under laboratory conditions (Experiments 1-10).

4.3 Incubation under hydrolytic conditions (Experiments 1, 2, 3, 5, 7, 9 and 10)

4.3.1 Solvent-extractable fractions

Figures 4.5 and 4.6 show the partial GC traces of the olive oil residues recovered during the experiments 1, 2, 3, 5, 7, 9 and 10 after a year of incubation. Before incubation (Figure 4.5a), olive oil consisted mainly of triacylglycerols (96%), ranging from C_{48} to C_{54}, with diacylglycerols only present as minor components (2.5%). During incubation, the triacylglycerols underwent hydrolysis to yield mainly free fatty acids but also mono- and diacylglycerols. As observed by Dudd and coworkers (1998) in experiments investigating the decay of olive oil, milk and pure triacylglycerols absorbed in unglazed ceramics, mono- and diacylglycerols were only produced in minor quantities.

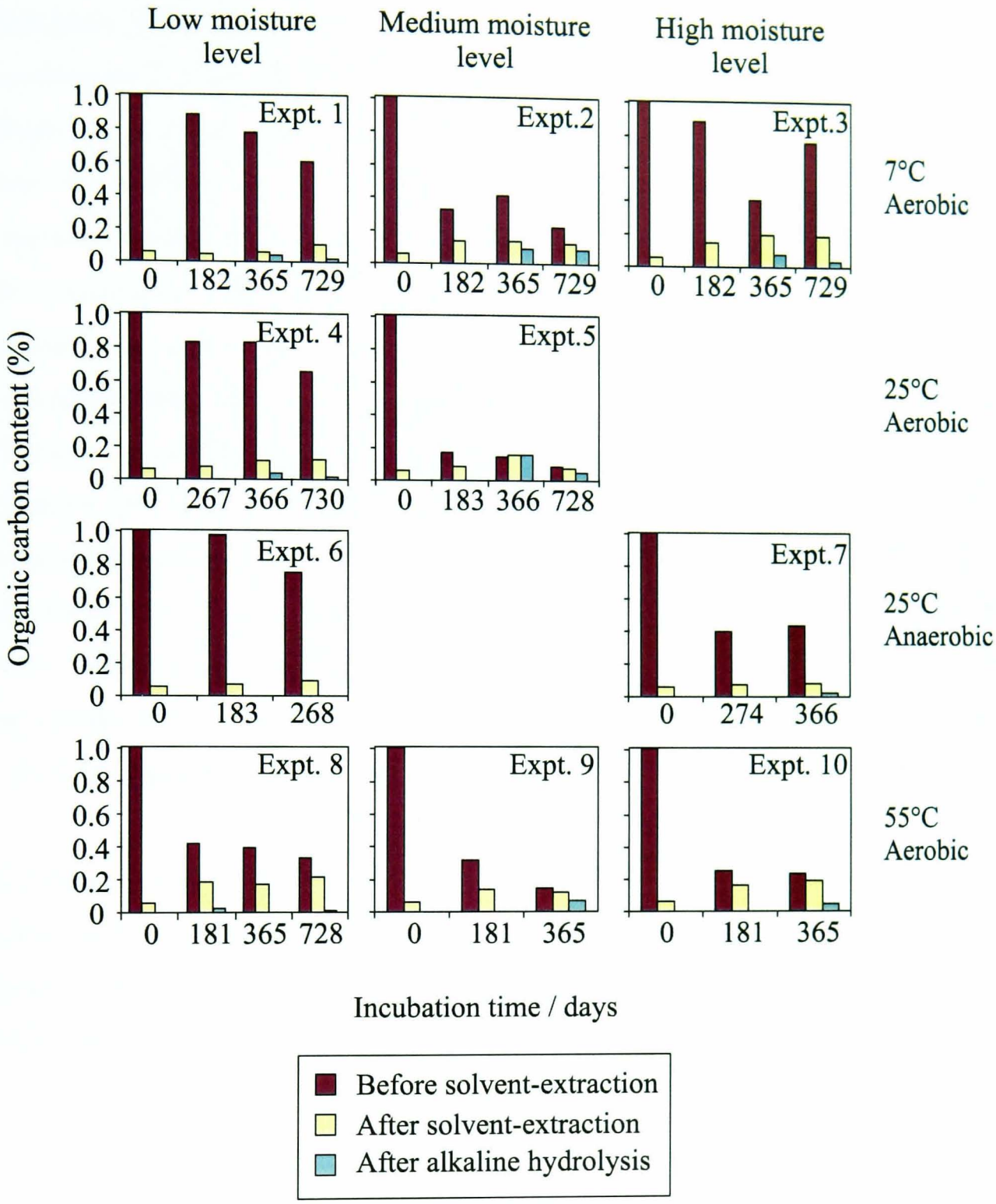


Figure 4.4: Potsherd organic carbon content before and after solvent-extraction, and after alkaline hydrolysis of the extracted ceramic, during the decay of olive oil under laboratory conditions (Experiments 1-10).

In most residues recovered during the experiments 1, 2, 3, 5, 7, 9 and 10, the triacylglycerol distributions, which will be studied in more detail in Section 4.3.1.1, remained similar to that observed in fresh olive oil. The residue recovered after a year of incubation under the conditions of the experiment 1 (Figure 4.5b) showed a broadened C_{54} peak, and rising of the baseline in the triacylglycerol elution range. As will be

explained in Section 4.4.1, this indicates that oxidation of the unsaturated acyl moieties took place, yielding more polar species which are not readily amenable to HTGC. During experiments 2, 3 and 5 (Figures 4.5c, 4.5d and 4.6a), the C_{54} triacylglycerol became very depleted and was not detected by HTGC. During the experiments 1, 2, 3, 5, and 7, the free fatty acid distribution was largely dominated by $C_{18:1}$ and was similar to that found in saponified fresh olive oil. The free fatty acid distribution of the residues recovered during the experiments 9 and 10 was altered as the $C_{18:1}$ fatty acid was preferentially depleted, probably because of its susceptibility to oxidation. A range of oxidation products, including hydroxy and dihydroxy octadecanoic acids, was also identified during these 2 experiments, and during the experiments 2, 5 and 7. The changes in the free fatty acid distributions and the oxidation products formed during the experiments conducted under hydrolytic conditions will be covered in Sections 4.3.1.2 and 4.3.1.3, respectively. During the experiment 5, an unknown compound accounted for 14.8% of the residue (Figure 4.6a). The mass spectrum of this compound analysed as its TMS ester and ether showed an intense ion at *m/z* 69 which could be $[CH_3CH_2CHC(CH_3)_2]^+$, but the complete structural assignment was not achieved.

β-5-Sitosterol was identified in 92% of the degraded olive oil residues. Its relative abundance accounted for 0.12 (Day 0) to 8.4% (Experiment 5, day 366) of the total extractable lipid. No sterol degradation products were identified during this set of experiments.

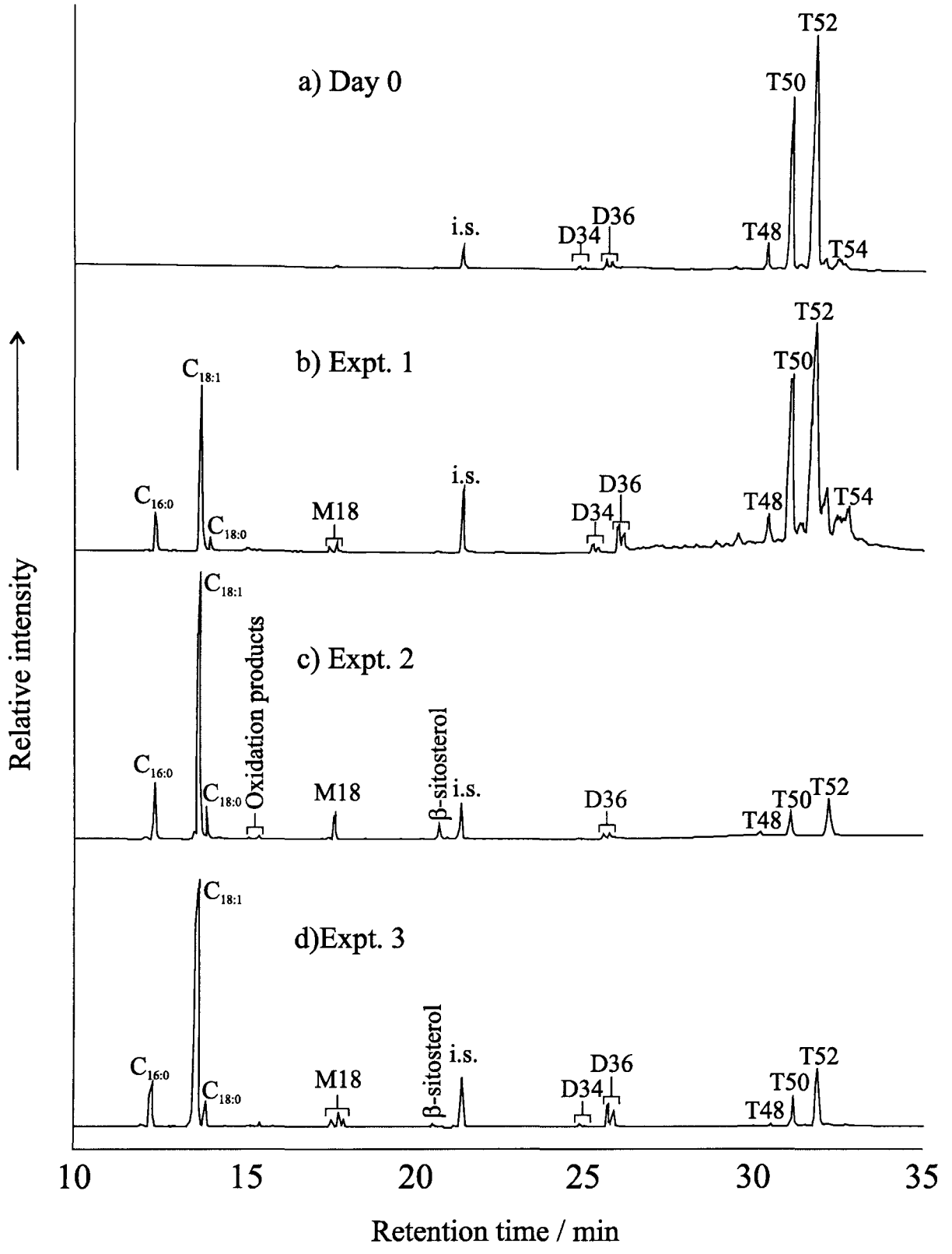


Figure 4.5: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions obtained after 0 day and one year of incubation during the experiments 1, 2 and 3. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; M18 = monoacylglycerol containing 18 acyl carbon atoms; i.s. = internal standard (*n*-tetratriacontane); D34 and D36 = diacylglycerols containing 34 and 36 acyl carbon atoms respectively; T48-T54 = triacylglycerols containing 48 to 54 acyl carbon atoms.

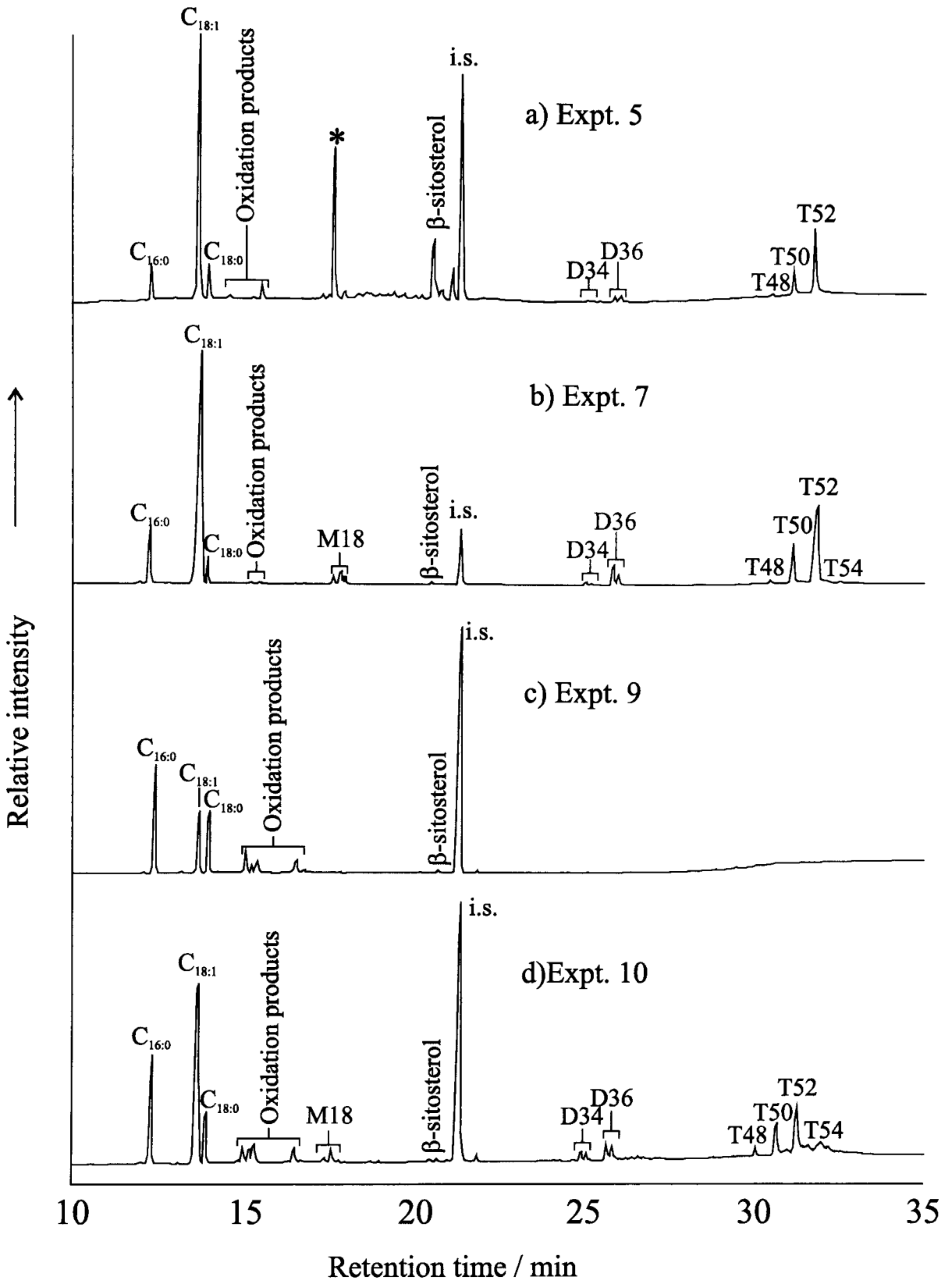


Figure 4.6: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions obtained after one year of incubation during the experiments 5, 7, 9 and 10. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; M18 = monoacylglycerol containing 18 acyl carbon atoms; * = unknown compound [*m/z* 69 (100), 81 (47), 95 (18), 121 (10)]; i.s. = internal standard (*n*-tetratriacontane); D34 and D36 = diacylglycerols containing 34 and 36 acyl carbon atoms respectively; T48-T54 = triacylglycerols containing 48 to 54 acyl carbon atoms.

4.3.1.1 Triacylglycerol distributions

The triacylglycerol distributions of the degraded olive oil residues recovered during experiments 1, 2, 3, 6, 7 and 10 after 1 and 2 years of decay are shown in Figure 4.7. The fresh olive oil used in these experiments contained the C_{48} (3.5%), C_{50} (27.5%), C_{52} (64.5%) and C_{54} (4.5%) triacylglycerols. The triacylglycerol distributions were not significantly altered until after 2 years of incubation by which time the C_{54} triacylglycerol had become so depleted that it was undetectable by HTGC (this was not observed in experiment 1). The relative abundances of the other triacylglycerols remained constant, displaying similar distributions to fresh olive oil. The range of relative abundances of each triacylglycerol during experiments 1, 2, 3, 6, 7 and 10 is shown in Table 4.4. In all experiments, the relative abundance of the C_{48} component varied between 0 and 6.5 % of the total triacylglycerol content. The relative abundance of the C_{50}, C_{52} and C_{54} components varied significantly, between 21.8 and 41.7%, 41.7 and 74.9% and 0 and 29.4%, respectively. The significant variation of the relative abundance of the C_{54} triacylglycerol is due to two factors: (i) after 1 to 2 years of incubation, the triacylglycerols became very degraded (see Section 4.3.1) to the point that C_{54} was not detectable any more by HTGC, and (ii) during the experiments 1, 6, and 7 a broadening of the C_{54} peak was observed (seen in Section 4.3.1 and treated in more detail in Section 4.4.1) very likely due to oxidation of the unsaturated fatty acyl moities.

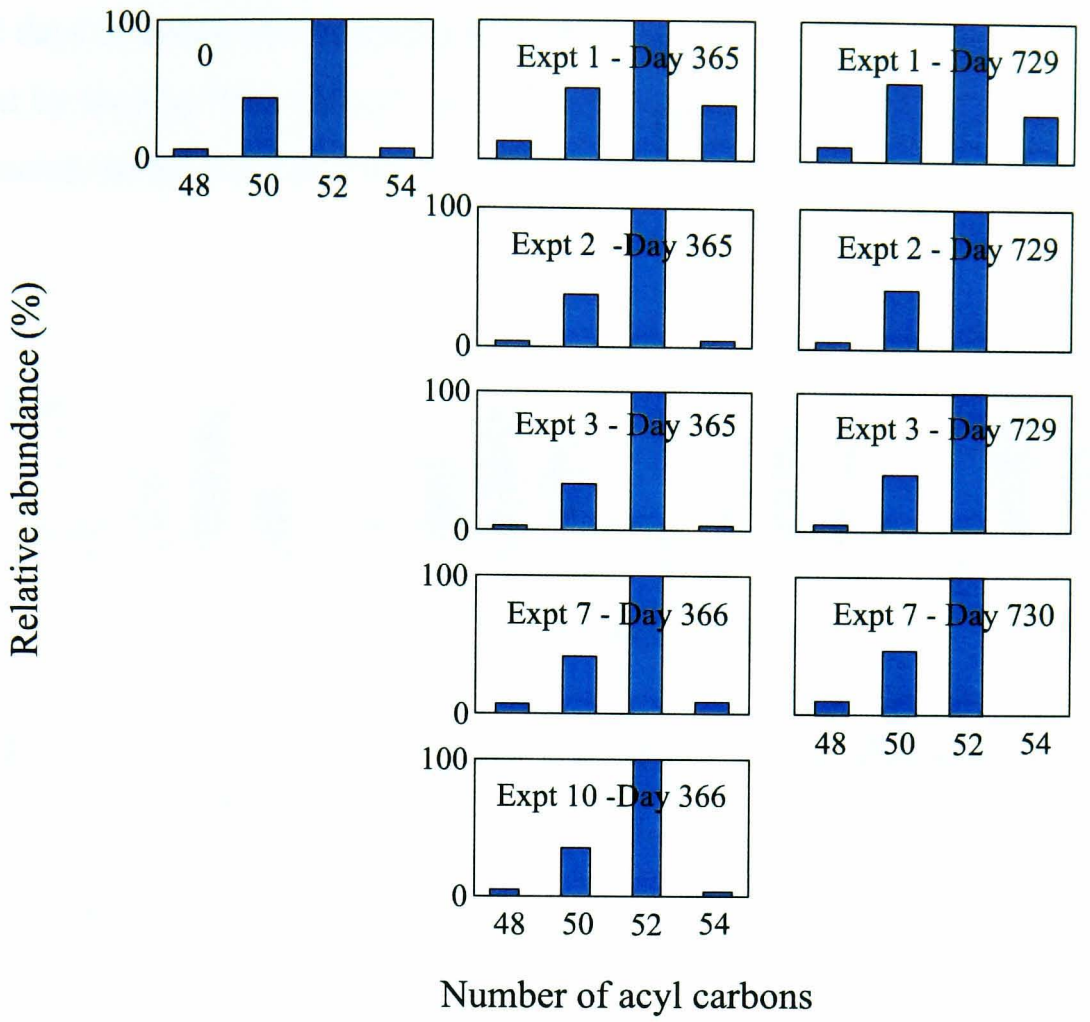


Figure 4.7: Triacylglycerol distribution in olive oil residues incubated for 1 and 2 years during the experiments 1, 2, 3, 7 and 10, compared with the triacylglycerol distribution of fresh olive oil.

Table 4.4: Triacylglycerol distributions during the laboratory decay of olive oil (Experiments 1, 2, 3, 6, 7 and 10).

	Relative abundance (%)			
	C_{48}	C_{50}	C_{52}	C_{54}
Experiment				
1	3.2 – 5.7	23.8 – 33.2	45.1 – 64.5	4.5 – 23.4
2	1.8 – 5.0	21.8 – 28.8	56.0 – 74.9	0 – 13.8
3	2.2 – 4.7	23.8 – 28.4	57.6 – 71.6	0 – 12.0
6	0 – 6.4	22.0 – 41.7	48.6 – 72.1	0 – 25.8
7	3.1 – 4.8	22.5 – 27.6	41.7 – 69.9	1.9 – 29.4
10	3.5 – 6.5	19.5 – 34.3	42.3 – 64.5	0 – 28.7

Figure 4.8 shows the triacylglycerol distributions after 23, 41, 61 and 132 days of incubation during the experiment 9. No triacylglycerols were detected after 132 days of incubation. The relative abundance of the C_{54} triacylglycerol increased from 4.5 (day 0) to 39.4% (day 61), again due to the broadening of the C_{54} peak possibly due to the presence of a co-eluting oxidation product of a lower carbon number triacylglycerol.

After 132 days of decay, the triacylglycerol distribution was very altered and had become dominated by the C_{50} (57.2%) and C_{52} (42.8%) components, with neither the C_{48} or C_{54} triacylglycerols being detectable by HTGC.

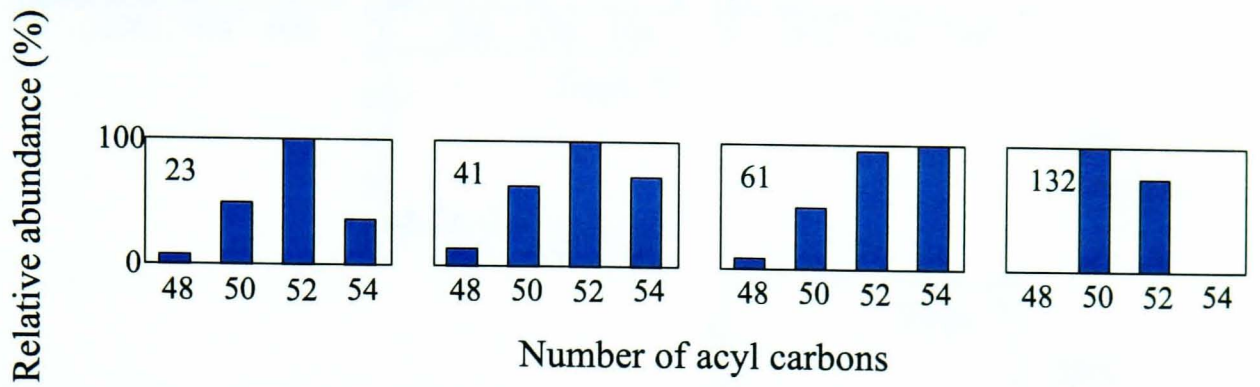


Figure 4.8: Triacylglycerol distributions in olive oil residues incubated for 23, 41, 61 and 132 days during the experiment 9.

4.3.1.2 Fatty acids

(i) Free fatty acid ratios

The changes in the free fatty acids ratios $C_{18:0}/C_{16:0}$ and $C_{18:1}/C_{16:0}$ during the experiments 1, 2, 3, 5, 7, 9 and 10 are shown in Figure 4.9. In all experiments conducted under "hydrolytic" conditions, the $C_{18:0}/C_{16:0}$ ratio remained constant and close to the value for saponified fresh olive oil ($C_{18:0}/C_{16:0}$ = 0.31 in the olive oil used for the experiments). Preferential decay by microorganisms of the $C_{16:0}$ fatty acid in the water of the burial environment is likely to be responsible for the slightly high value observed during the experiment 5 (1.04 after 366 days of incubation). The ratio $C_{18:1}/C_{16:0}$ was always found to be lower than the value for the fresh olive oil (7.70 in the olive oil used for these experiments), probably because of the susceptibility of the $C_{18:1}$ fatty acid to oxidative degradation. This was especially noticeable in the experiments 9 and 10, where the $C_{18:1}/C_{16:0}$ ratio was 0.6 and 3.0 respectively after 1 year of incubation. The high temperatures (55° C) at which these incubations were incubated appears to promote the degradation of the $C_{18:1}$ fatty acid.

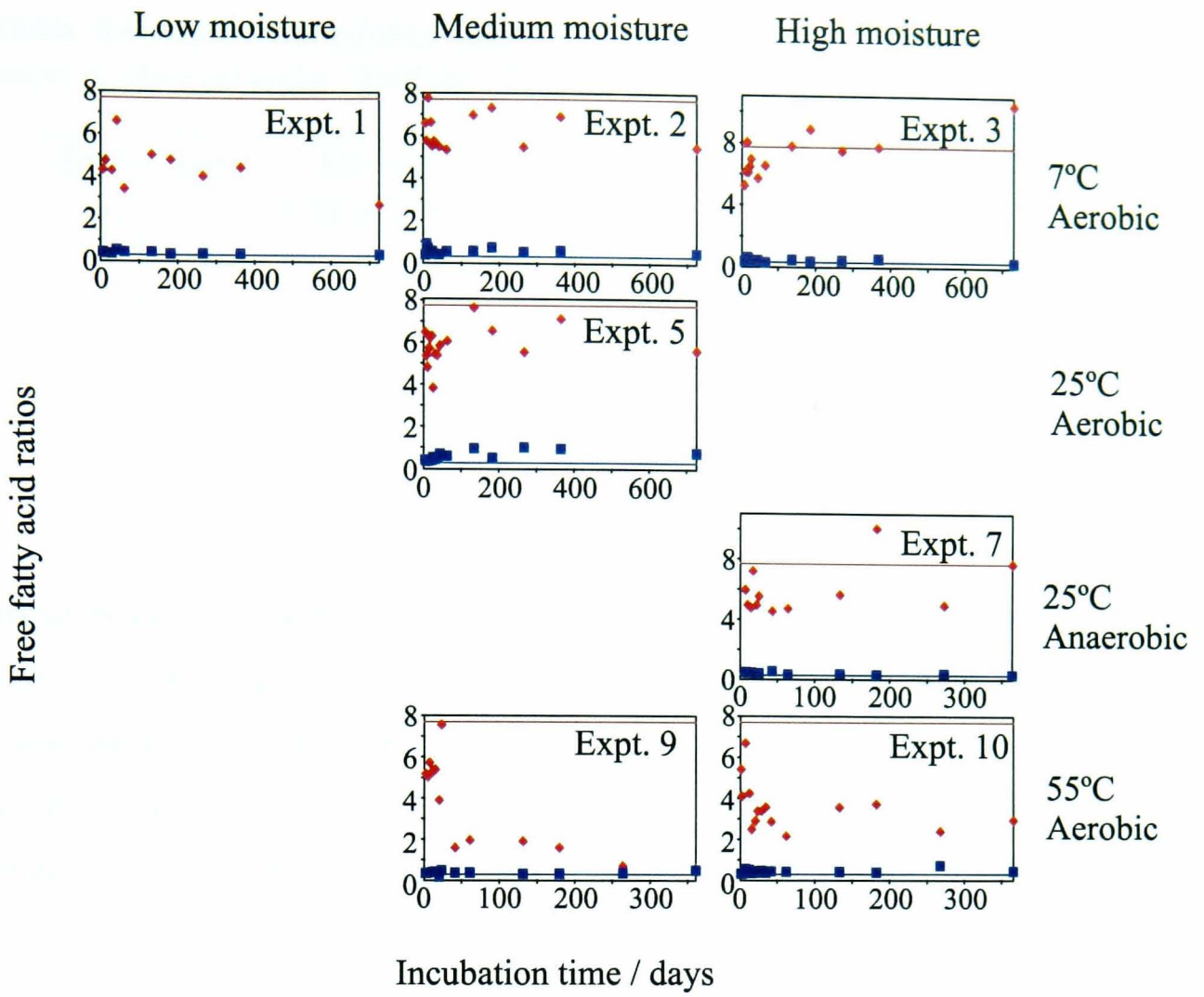


Figure 4.9: Changes in the $C_{18:1}/C_{16:0}$ (◆) and $C_{18:0}/C_{16:0}$ (■) ratios during the laboratory decay of olive oil under "hydrolytic" conditions (experiments 1, 2, 3, 5, 7, 9 and 10), compared with the $C_{18:1}/C_{16:0}$ (—) and $C_{18:0}/C_{16:0}$ (—) ratios in the commercial olive oil used as a substrate.

(ii) Bacterial fatty acids

The straight-chain and branched free fatty acids $C_{17:0}$ were detected at low abundance in olive oil residues recovered from experiments 1, 2, 3, 5, 7, 9 and 10 (Table 4.5). These free fatty acids and the straight-chain and branched $C_{15:0}$ have been previously identified by Dudd *et al.* (1998) in degraded residues resulting from the experimental decay of olive oil and pure triacylglycerols absorbed in unglazed ceramics, and have been identified as bacterial contaminants, albeit at very low concentrations (Ratledge and Wilkinson, 1988). Dudd and coworkers (1998) also reported the presence of ergosterol in degraded milk residues absorbed in unglazed ceramics, thought to arise from fungal growth.

Table 4.5: Maximum relative abundance of the $C_{17:0}$ fatty acid during the laboratory decay of olive oil under "hydrolytic" conditions (Experiments 1, 2, 3, 5, 7, 9 and 10).

Experiment	Incubation temperature / °C	Moisture level	Aeration conditions	Day	Total $C_{17:0}$ (%)
1	7	Low	Aerobic	729	0.2
2	7	Medium	Aerobic	729	0.6
3	7	High	Aerobic	365	0.4
5	25	Medium	Aerobic	14	0.2
7	25	High	Anaerobic	22	0.2
9	55	Medium	Aerobic	364	0.6
10	55	High	Aerobic	132	0.2

Incorporation of significant quantities of bacterially-derived components in unglazed ceramics could prevent the correct identification of degraded residues as these components would alter the chemical or isotopic composition of the residues. In the experiments described herein, bacterial contribution to the residue remained minor even if the incubation conditions were chosen so as to promote microbial activity.

(iii) Double-bond position and configuration in $C_{18:1}$

DMDS adducts of the unsaturated $C_{18:1}$ fatty acids in selected degraded olive oil residues were prepared as described in Section 2.5.4 and analysed by GC-MS as described in Section 2.6.2 to monitor the position and configuration of the double-bond. Olive oil was found to contain almost exclusively oleic acid (97.7%), with the double-bond at the carbon 9 and in a *cis*-configuration. Two other isomers were detected in minor quantities: *trans*-$C_{18:1}\Delta^9$ (0.4%) and *cis*-$C_{18:1}\Delta^{11}$ (1.9%). No significant difference was observed in the degraded residues recovered at the beginning and end of the experiments as all showed oleic acid as the main component (> 95%).

4.3.1.3 Oxidation products

Table 4.6 shows the maximum relative abundance of the oxidation products identified during experiments 1, 2, 3, 5, 7, 9 and 10. During experiment 9 (Figure 4.10), the relative abundance of the oxidation products increased rapidly from 0 (day 0) to 50% (day 132), after which it decreased to 15% after 264 days, as oxidation products either were degraded to form volatile products or became irreversibly "bound" to the ceramic matrix

so that they were not detectable by HTGC.

Table 4.6: Maximum relative abundance of the oxidation products recovered during the experiments 1, 2, 3, 5, 7, 9 and 10 investigating the decay of olive oil under "hydrolytic" conditions.

Expt.	Incubation temperature / ° C	Moisture level	Aeration conditions	Day	Total oxidation products (%)
1	7	Low	Aerobic	729	4.8
2	7	Medium	Aerobic	267	5.7
3	7	High	Aerobic	267	9.3
5	25	Medium	Aerobic	22	9.0
7	25	High	Anaerobic	64	6.9
9	55	Medium	Aerobic	132	49.2
10	55	High	Aerobic	7	15.4

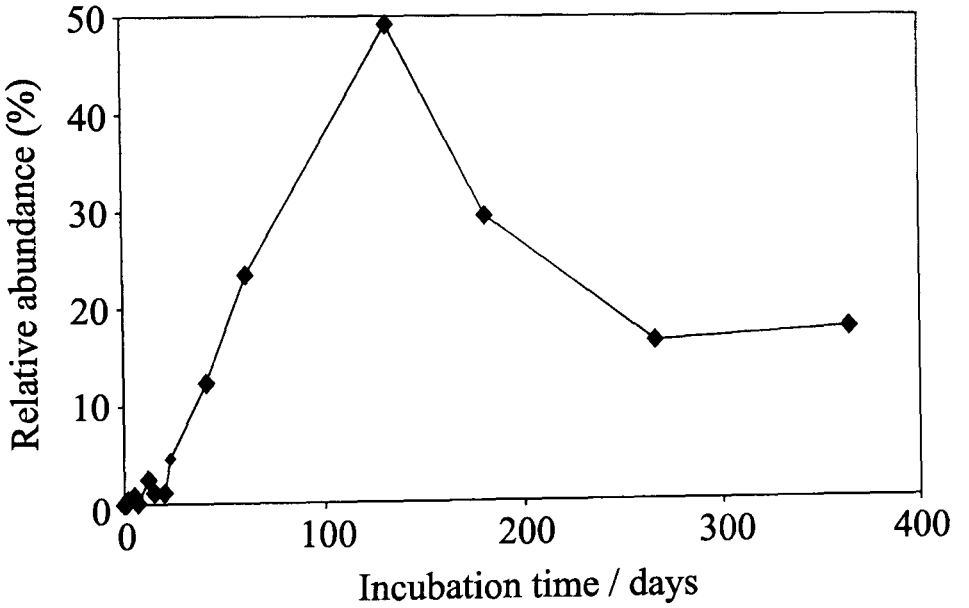


Figure 4.10: Changes in the relative abundance of the oxidation products during experiment 9 investigating the decay of olive oil under laboratory conditions.

All samples contained a similar range of same oxidation products, namely mono- and dihydroxy compounds containing 18 carbon atoms, in very similar proportions. Figure 4.11 shows as an example the partial HTGC trace of the residue recovered after 132 days of incubation during experiment 10. A similar range of compounds was previously reported in olive oil residues degraded under laboratory conditions (Dudd, 1999).

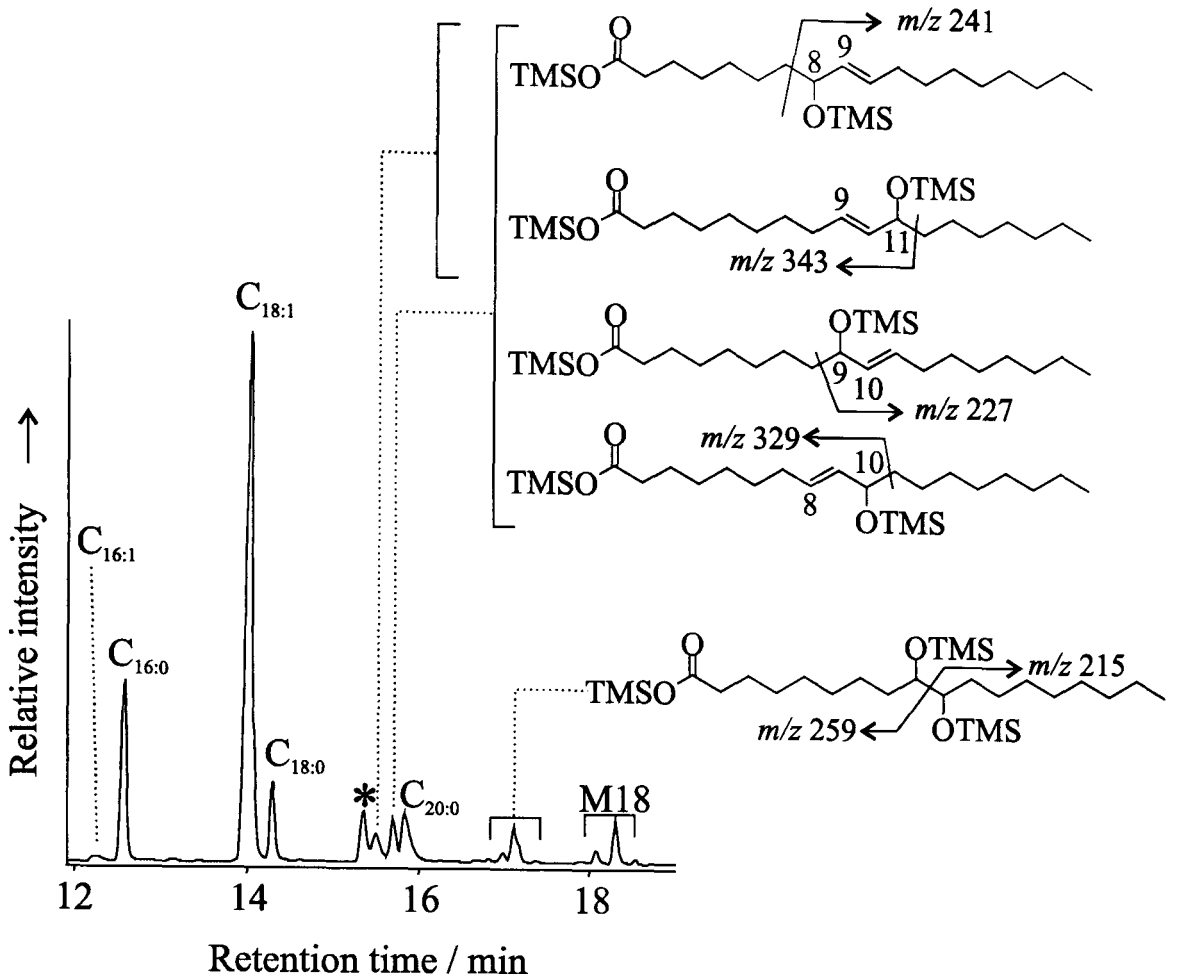


Figure 4.11: Partial HTGC trace of the trimethylsilylated solvent-extractable fraction of an olive oil residue incubated for 132 days during the experiment 10. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; * = unknown compound [*m/z* 73 (100), 117 (58), 155 (38), 213 (20), 229 (15), 337 (30)]; M18 = monoacylglycerol containing 18 acyl carbon atoms.

4.3.2 "Bound" fractions

As was described in Section 4.2.2, a significant amount of organic carbon remained associated with the potsherd after solvent-extraction. Alkaline hydrolysis was therefore performed on the extracted potsherd as described in Section 2.4.2.3. The released compounds were methylated (Section 2.5.3) and trimethylsilylated (Section 2.5.5) and analysed by GC on a CPSil5 column (Section 2.6.1). Partial GC trace of the "bound" residue recovered after 366 days of incubation during the experiment 7 is shown in Figure 4.12 and the composition of the "bound" residues formed during experiments 1, 3, 5, 7, 9 and 10 after a year of incubation is shown in Table 4.7. All residues were dominated by the $C_{16:0}$ and $C_{18:0}$ fatty acids in the same proportions as those found in saponified fresh olive oil. The unsaturated $C_{18:1}$ was significantly depleted compared to fresh olive oil. The range of oxidation products was identified in the "bound" residues, including α,ω-dicarboxylic acids, and hydroxy acids. The nature of the "binding"

between the compounds released after alkaline hydrolysis and the ceramic matrix is not well understood. It is thought that the compounds identified in the "bound" fraction are either (i) strongly associated with the mineral phase of the ceramic matrix *via* salt or dipole-dipole interactions, or (ii) part of a highly polymerised network of polar triacylglycerols.

4.4 Incubation under oxidative conditions (Experiments 4, 6 and 8)

4.4.1 Solvent-extractable fractions

Figures 4.13 to 4.15 shows partial HTGC traces of solvent-extractable fractions recovered during the experiments 4, 6 and 8 conducted under oxidative conditions. Their composition is detailed in the following sections.

4.4.1.1 Triacylglycerols

During the experiments 4, 6 and 8, the triacylglycerols became reduced in abundance very rapidly. Their distribution was very altered as the triacylglycerol peaks became very broad and overlapped (Figures 4.13b, c and d; 4.14a, b and c; 4.15a). The base line rose significantly in the retention time range of the triacylglycerols, revealing the presence of polar compounds eluting in this area. The formation of a complex mixture of high molecular weight lipids (Rt = 22 - 35 min), unresolvable by high temperature gas chromatography (HTGC) analysis, was observed, particularly during experiments 4 (Figure 4.13d) and 8 (Figure 4.15a). Mass spectra studied along the GC profile revealed the presence of intact triacylglycerols and diacylglycerols within the complex mixture.

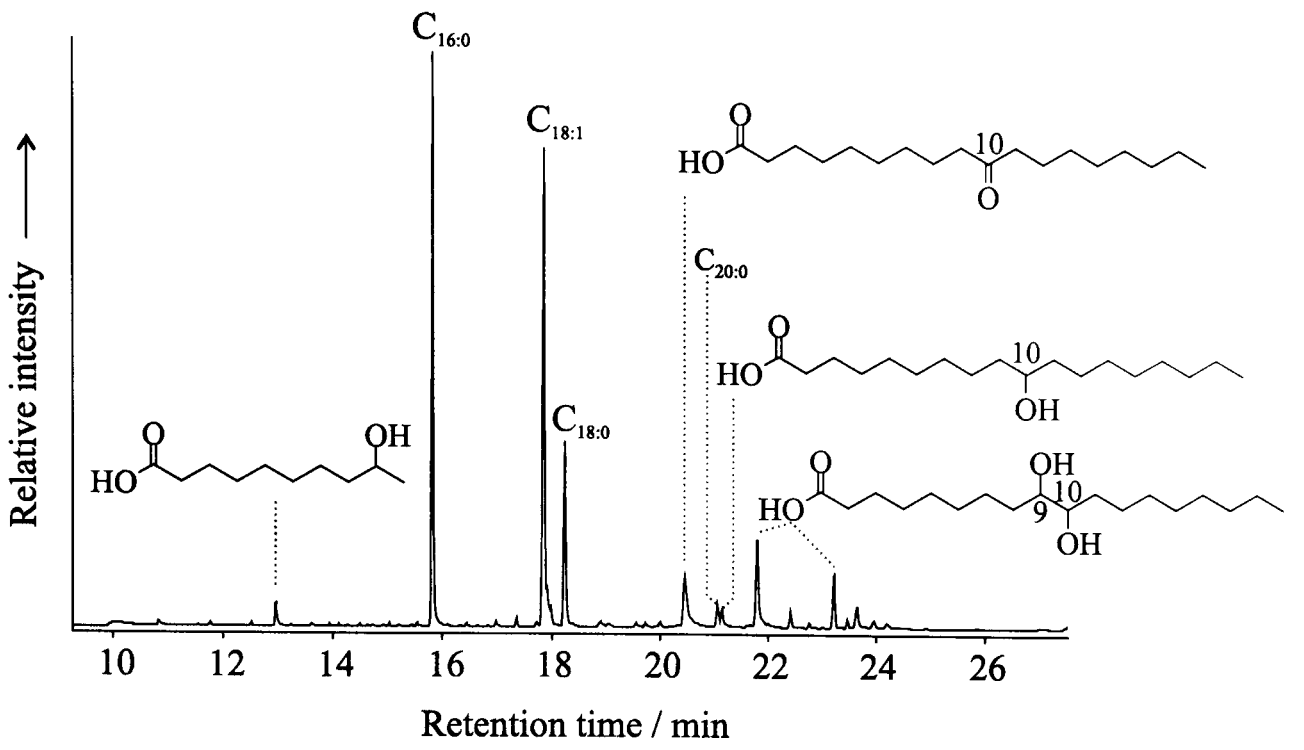


Figure 4.12: Partial GC trace of the "bound" residue recovered after 366 days of incubation during the experiment 7. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds present as their methyl esters. All other compounds are present as their Me esters TMS ethers.

Table 4.7: Composition of the "bound" fractions recovered from experiments 1, 3, 5, 7, 9 and 10 investigating the decay of olive oil under "hydrolytic" conditions.

	Relative abundance (%)					
Experiment	1	3	5	7	9	10
Day	365	365	366	366	364	365
Saturated fatty acids						
$C_{16:0}$	28.7	23.3	18.4	24.8	40.5	39.7
$C_{18:0}$	11.6	14.1	13.4	10.1	22.67	19.0
$C_{20:0}$	2.7	2.8	2.3	1.3	2.5	4.3
$C_{22:0}$	0.4	-	2.6	-	-	-
$C_{24:0}$	-	-	2.1	-	-	-
Unsaturated fatty acids						
$C_{16:1}$	0.3	1.4	-	-	1.2	1.4
$C_{18:1}$	33.4	28.9	-	25.6	-	25.3
Hydroxy acids						
2-hydroxydecanoic acid	-	2.5	-	-	-	-
9-hydroxydecanoic acid	1.8	0.5	1.4	1.3	-	1.5
2-hydroxydodecanoic acid	-	-	2.9	-	-	-
10-hydroxyoctadecanoic acid	-	-	-	1.1	-	-
9,10-dihydroxyoctadecanoic acid	2.7	3.1	3.2	12.0	14.4	3.9
Keto-acids						
9-oxo-decanoic acid	3.3	3.4	1.5	-	-	-
10-oxo-decanoic acid	-	-	1.6	5.5	-	-

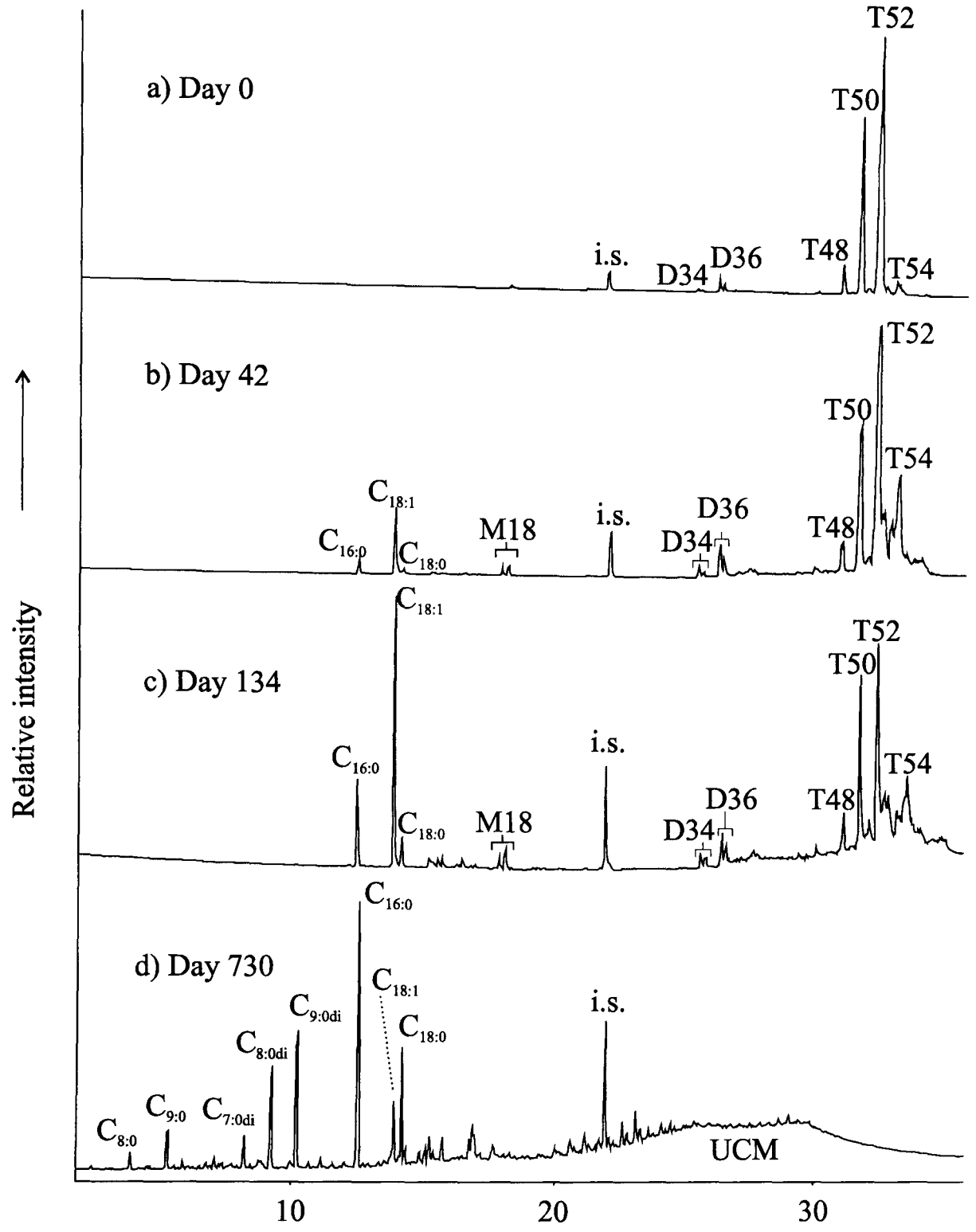


Figure 4.13: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions recovered after a) 0, b) 42, c) 134 and d) 730 days of incubation during the experiment 4. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; M18 = monoacylglycerol containing 18 acyl carbon atoms; i.s. = internal standard (*n*-tetratriacontane); D34 and D36 = diacylglycerols containing 34 and 36 acyl carbon atoms respectively; T48 – T54 = triacylglycerols containing 48 to 54 acyl carbon atoms; $C_{m:ndi}$ = α,ω-dicarboxylic acids containing m carbon atoms and n double-bonds; UCM = unresolved complex mixture.

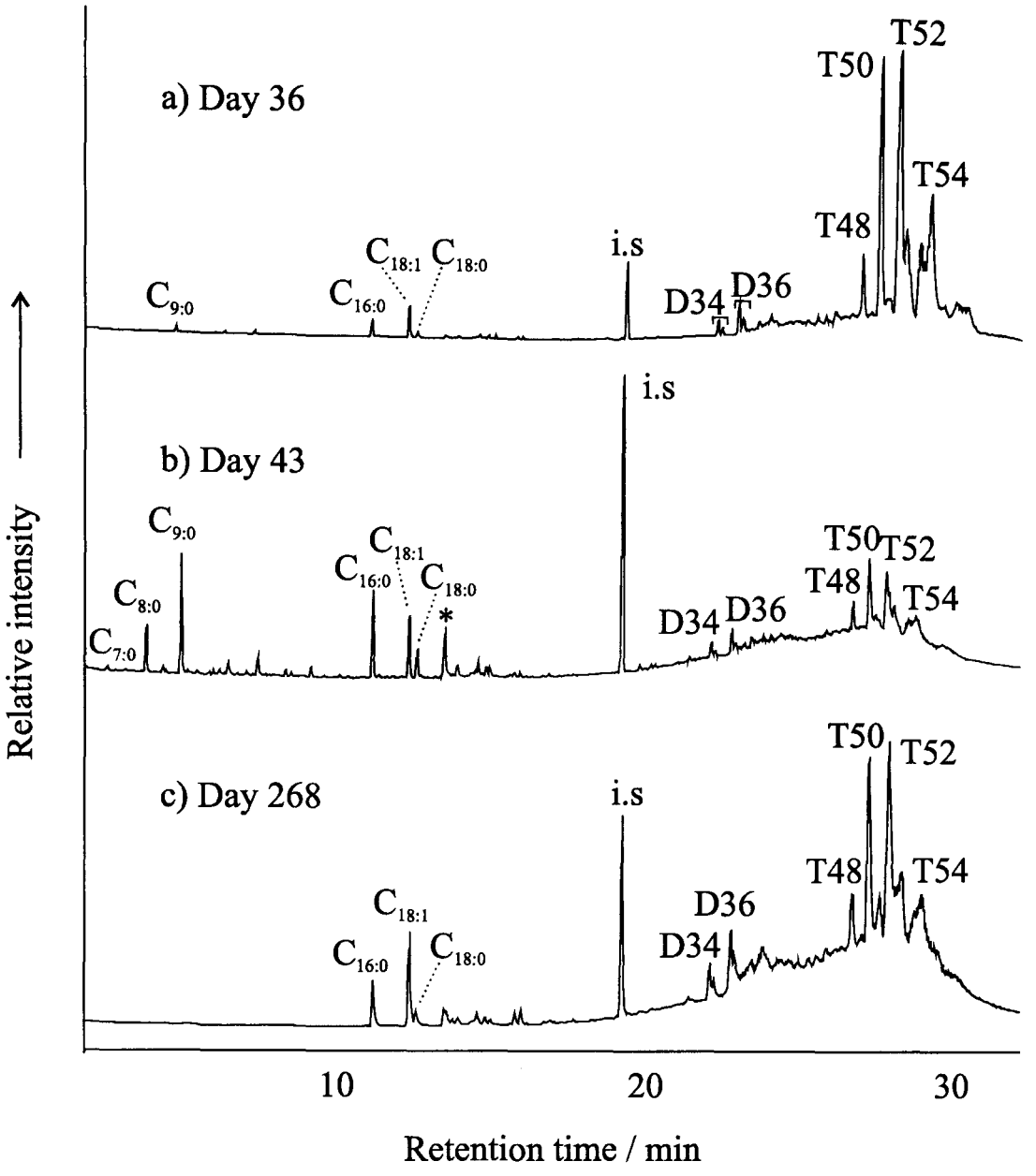


Figure 4.14: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions recovered after a) 36, b) 43 and c) 268 days of incubation during the experiment 6. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; i.s. = internal standard (*n*-tetratriacontane); D34 and D36 = diacylglycerols containing 34 and 36 acyl carbon atoms respectively; T48 – T54 = triacylglycerols containing 48 to 54 acyl carbon atoms.

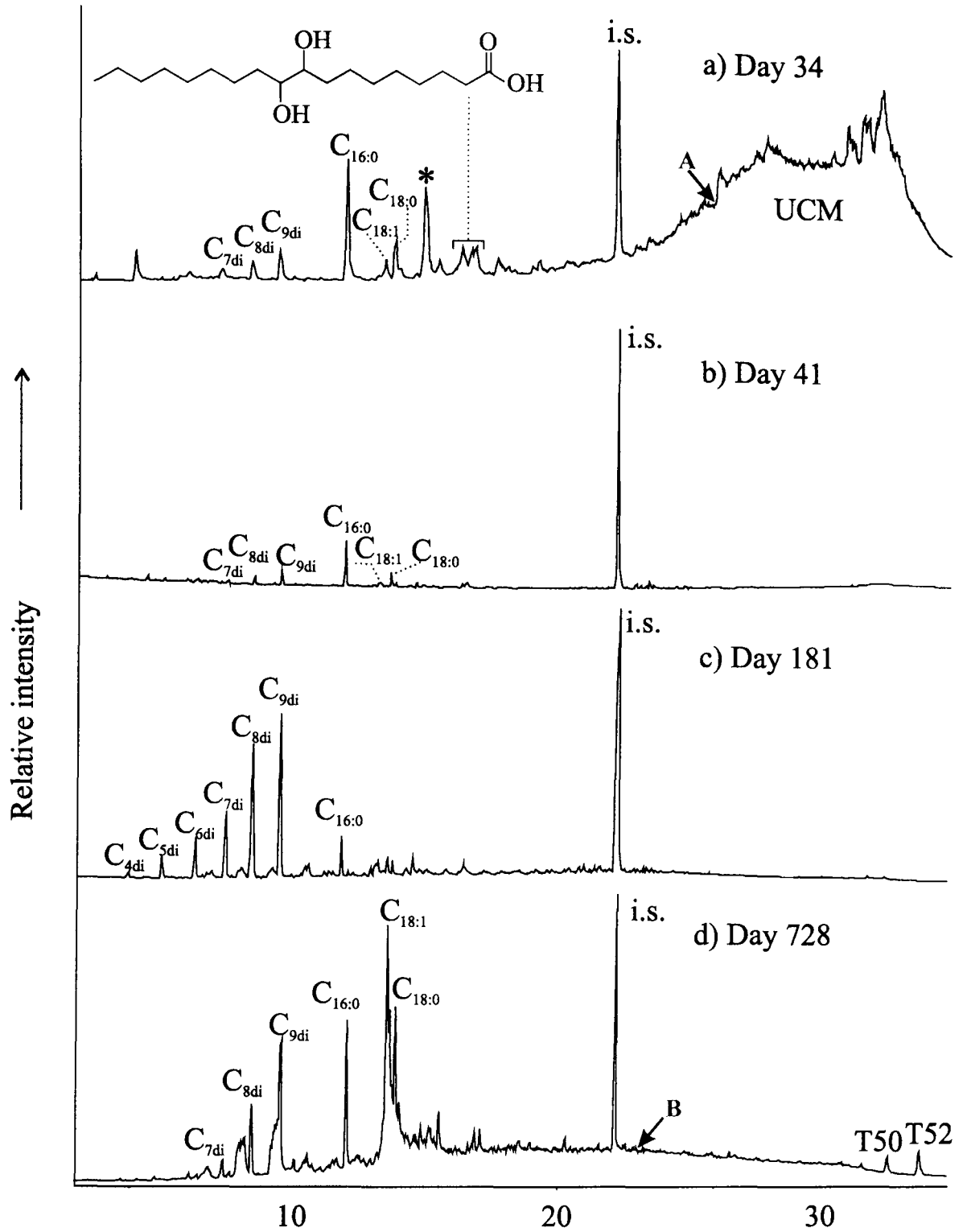


Figure 4.15: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions recovered after a) 34, b) 41, c) 181 and d) 728 days of incubation during the experiment 8. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; i.s. = internal standard (*n*-tetratriacontane); T50 and T52 = triacylglycerols containing 50 and 52 acyl carbon atoms respectively; C_{xdi} = α,ω-dicarboxylic acids containing x carbon atoms; UCM = unresolved complex mixture; * = unknown peak [*m/z* 73 (100), 117 (58), 155 (38), 213 (20), 229 (15), 337 (30)].

Figure.4.16a shows the typical mass spectrum at the retention time **A** indicated in Figure 4.15a. It contained strong ions at *m/z* 73 $[(CH_3)_3Si]^+$, 74 $[(CH_3)_2SiOH]^+$ and 117 $[(CH_3)_3SiOC(OH)CH_2]^+$, indicative of the presence of trimethylsilylated carboxyl or hydroxyl functions that are not normally encountered in the mass spectra of triacylglycerols. The weak ions at *m/z* 267 $[C_{18}H_{35}O]^+$ and 313 $[C_{16}H_{31}O^+ + 74]$, arose from intact acyl moieties of stearate and palmitate, respectively, esterified to the glycerol backbone. The ions *m/z* 129 $[CH_2CHCHOSi(CH_3)_3]^+$ and 145 $[CH(O)CHCH_2OSi(CH_3)_3]^+$ are both commonly encountered in the mass spectra of diacylglycerols. The ion at *m/z* 215 $[C_9H_{18}OSi(CH_3)_3]^+$ was indicative of the presence of an acyl moiety hydroxylated at position 9. These fragments are indicative of the presence of oxidised moieties esterified to the glycerol backbone, and are consistent with the tentative structure shown in Figure 4.16a.

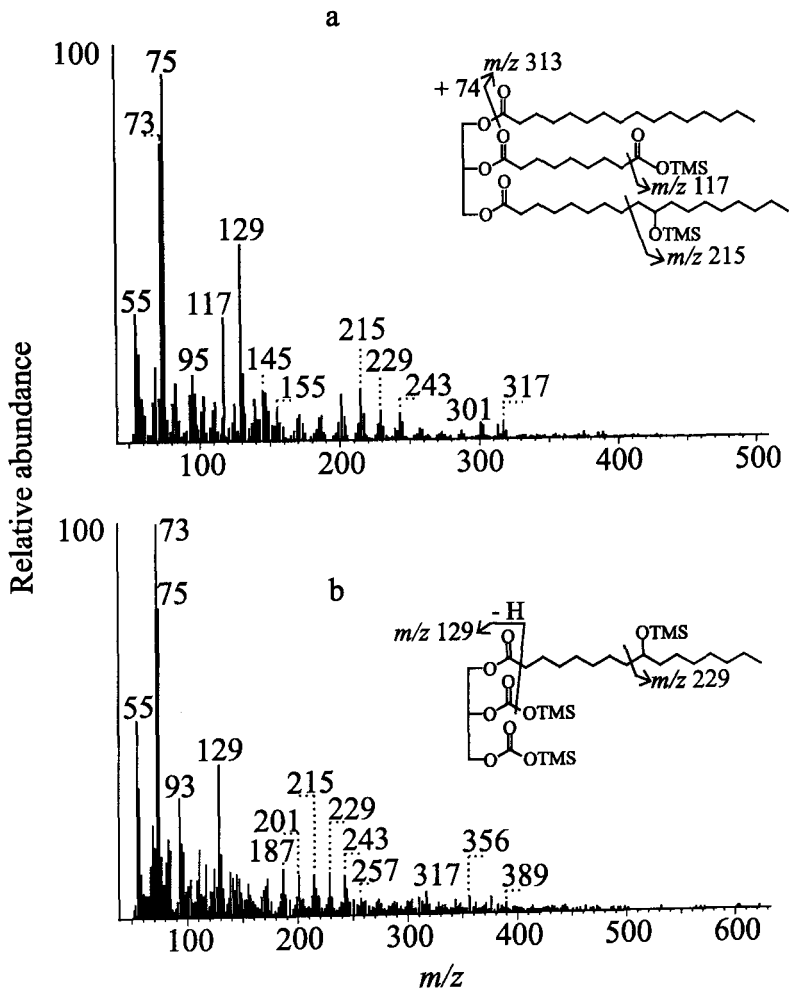


Figure 4.16: Mass spectral fragmentation recorded at the retention times a) **A** shown in Figure 4.15a, and b) **B** shown in Figure 4.15d.

During the experiment 8, the high molecular weight UCM was not observed in the later stages of the experiment. However, after 728 days of incubation (Figure 4.15d), the partial GC trace of the residue showed a raised base line between Rt = 8 to 30 min, and chromatographic resolution of the free fatty acids peaks was reduced, as peaks were broad and overlapped (Rt = 8 – 16 min). Figure 4.16b shows a typical mass spectrum at the retention time **B** in Figure 4.15d. It was dominated by ions at *m/z* 73, 74 and 129, characteristic of mono- and diacylglycerols. The ion *m/z* 117 was not present in significant quantities. Ions characteristics of mid chain hydroxyl groups, such as *m/z* 201, 215, 229 and 243, were present. These fragments are characteristic of mono- and diacylglycerols whose fatty moieties contain a hydroxyl function, as shown in the tentative structure in the Figure 4.16b. The structures in Figures 4.16a and 4.16b showing oxidised acyl moieties esterified to the glycerol backbone are consistent with previous work investigating the experimental oxidation of triolein (Neff and Byrdwell, 1998; Byrdwell and Neff, 1999).

4.4.1.2 Fatty acids

(i) Free fatty acid ratios

During experiments 4, 6 and 8, partial hydrolysis of the triacylglycerols occurred, yielding mainly palmitic acid ($C_{16:0}$), and minor quantities of stearic and oleic acids ($C_{18:0}$ and $C_{18:1}$, respectively; Figure 4.13, Figure 4.14 and Figure 4.15). The commercial olive oil used in these experiments contained 79% $C_{18:1}$ which should therefore be the principal fatty acid released by hydrolysis. The unexpected dominance of $C_{16:0}$ over $C_{18:1}$ was observed during most of the experiments.

Figure 4.17 shows the changes in the ratios of $C_{18:1}$ and $C_{18:0}$ to $C_{16:0}$ during experiments 4, 6 and 8. During these experiments the $C_{18:0}/C_{16:0}$ ratio remained constant and similar to the value obtained for saponified fresh olive oil. The $C_{18:1}/C_{16:0}$ ratio, however, was lower than the value for saponified fresh olive oil, and decreased very rapidly during these 3 experiments until it became equal to the $C_{18:0}/C_{16:0}$ ratio. During the experiments 4 and 8, the $C_{18:1}/C_{16:0}$ ratio remained low throughout the experiments, but, during the experiment 6, it first decreased to *ca.* 2 after 37 days of incubation, then increased back to 6 by 183

days of incubation, but then decreased to 2 after 268 days of incubation.

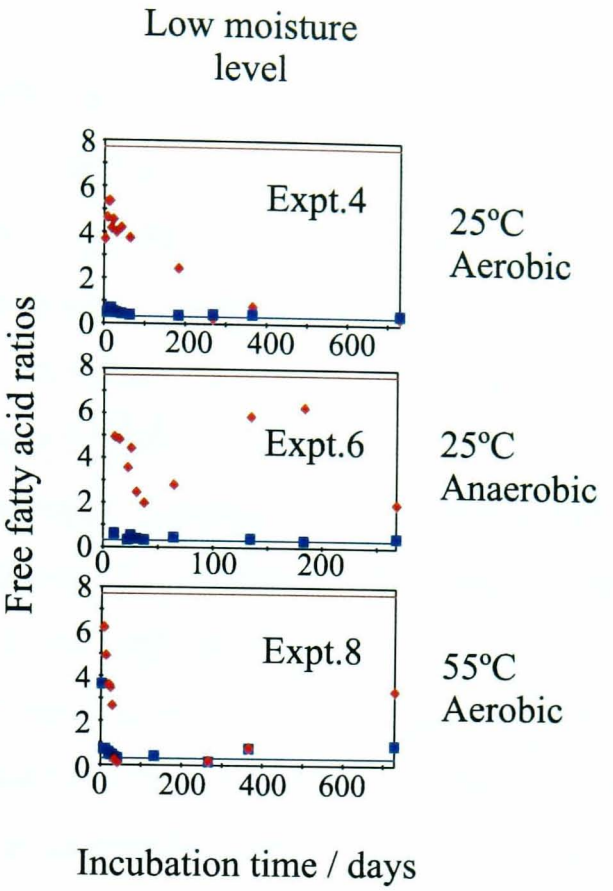


Figure 4.17: Changes in the $C_{18:1}/C_{16:0}$ (◆) and $C_{18:0}/C_{16:0}$ (■) ratios during the laboratory decay of olive oil under "oxidative" conditions (experiments 4, 6 and 8), compared with the $C_{18:1}/C_{16:0}$ (—) and $C_{18:0}/C_{16:0}$ (—) ratios in the commercial olive oil used as a substrate.

(ii) Bacterial fatty acids

No bacterial fatty acids (branched $C_{15:0}$ and $C_{17:0}$) were detected during experiments 4, 6 and 8, probably because the conditions under which these experiments were conducted were not favourable to bacterial growth.

(iii) Double-bond position and configuration in $C_{18:1}$

Similar to what was observed during the experiments conducted under "hydrolytic" conditions (Section 4.3.1.2) the double-bond position and configuration in $C_{18:1}$ isomers in the degraded residues obtained during the experiments 4, 6 and 8 were very similar to that observed in fresh olive oil and were dominated by oleic acid (> 95%).

4.4.1.3 Oxidation products

Quantification of the oxidation products present in the residues recovered during the experiments 4, 6 and 8 was difficult because of the lack of chromatographic separation between polar compounds (e.g. Figures 4.13, 4.14 and 4.15). It was however possible to estimate the relative abundance of hydroxy and dicarboxylic acids as shown in Figure 4.18. Oxidation products were most abundant during experiment 8 (conducted at 55°C), during which their relative abundance rapidly increased to 78% after 286 days of incubation and then decreased to 40% after 730 days of incubation. During experiment 4 (conducted at 25° C under aerobic conditions), the relative abundance of the oxidation products increased to 40% and 30% after 365 and 730 days of incubation, respectively. During the experiment 6 (conducted at 25° C under anaerobic conditions), oxidation products accounted for less than 5% of the residues. Based on these observations, it is possible to conclude that high temperatures and the presence of air favour the formation of oxidation products.

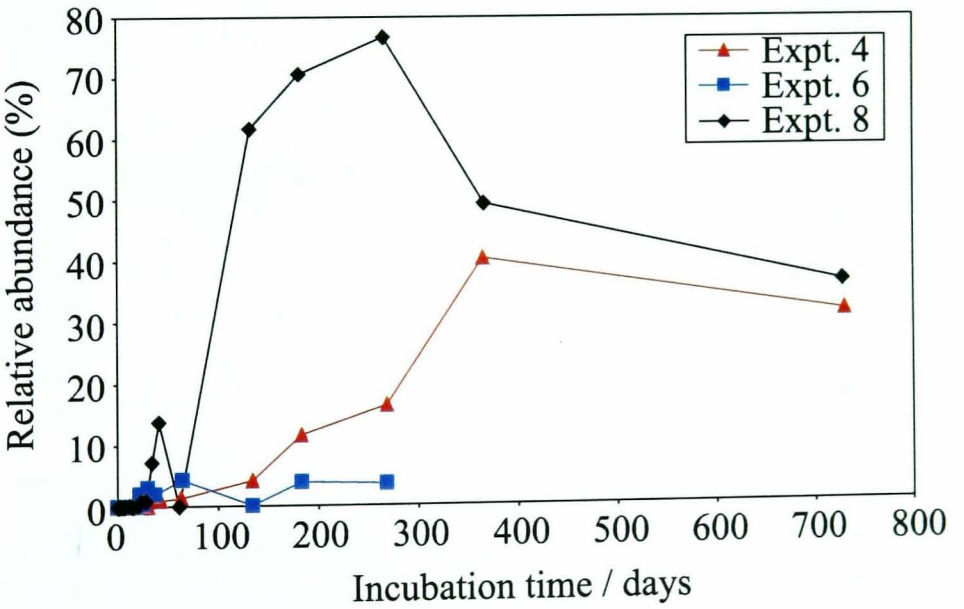


Figure 4.18: Changes in the relative abundance of the hydroxy and dicarboxylic acids during the laboratory decay of olive oil under "oxidative" conditions (Experiments 4, 6 and 8).

The oxidation products detected included α,ω-dicarboxylic acids, ranging from C_4 to C_9, dominated by C_9 (Figures 4.13 and 4.15), short-chain fatty acids, ranging from C_7 to C_9, dominated by C_9 (Figures 4.13 and 4,14) and 9, 10-dihydroxyoctadecanoic acid (Figure

4.15a). Polar triacylglycerols and diacylglycerols, unresolvable by HTGC, were not quantified.

4.4.2 "Bound" fractions

The "bound" fractions recovered from experiments 4, 6 and 8 all contained the same compounds previously observed in the solvent extracts (Table 4.8). They comprised fatty acids ranging from $C_{16:0}$ to $C_{20:0}$ or $C_{22:0}$, a series of α,ω-dicarboxylic acids ranging from C_7 to C_{11}, with the C_9 component the most abundant, 9-hydroxy decanoic acid and 9,10-dihydroxy octadecanoic acid.

Table 4.8: Composition of the "bound" fraction recovered after 2 years of incubation during experiments 4 and 8.

		Relative abundance (%)	
	Experiment	4	8
Compounds	Day	730	728
Saturated fatty acids			
$C_{16:0}$		28.8	6.6
$C_{18:0}$		10.0	2.3
$C_{20:0}$		1.5	0.2
$C_{22:0}$		0.2	-
α,ω-dicarboxylic acids			
$C_{7:0}$		1.0	1.6
$C_{8:0}$		14.5	14.4
$C_{9:0}$		20.9	31.7
$C_{10:0}$		1.7	5.7
$C_{11:0}$		-	2.6
Hydroxy acids			
9-hydroxydecanoic acid		6.0	3.2
9,10-dihydroxyoctadecanoic acid		1.8	0.8

The composition of the "bound" fractions recovered during experiments 4,6 and 8 changed with time, as shown in Figure 4.19 which shows the partial GC traces of the "bound" fractions recovered during the experiment 8. After 34 days of incubation, the "bound" fraction was dominated by the $C_{16:0}$ and $C_{18:0}$ fatty acids, but after 1 year, and more especially after 2 years of incubation, the relative abundance of the fatty acids decreased and the residues became dominated by the α,ω-dicarboxylic acids. Figure 4.19

shows that the composition of the "bound" fraction changes with time showing an increased abundance of oxidation products as the experiment progresses.

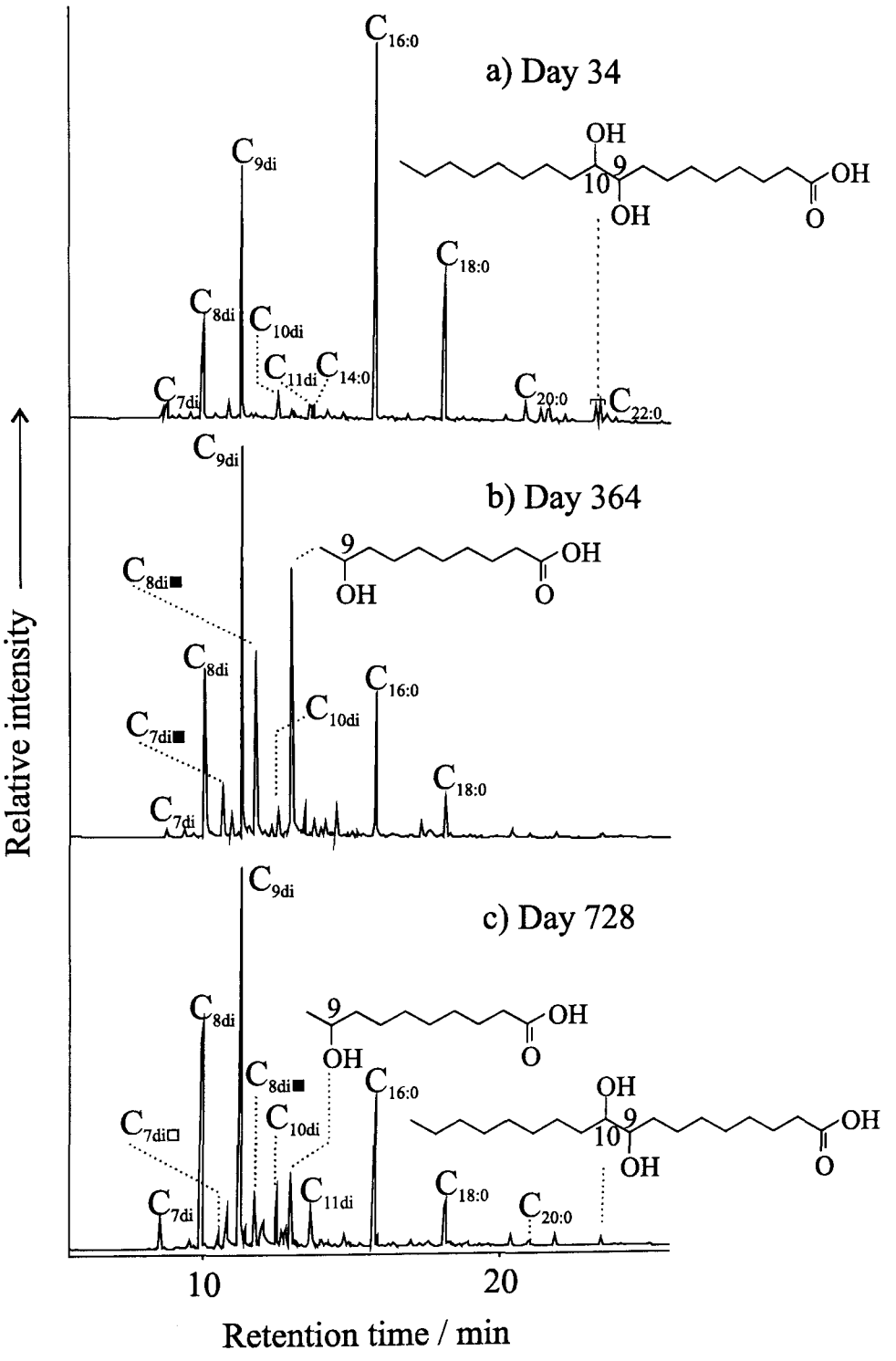


Figure 4.19: Partial GC profile of the "bound" lipid fraction recovered after alkaline hydrolysis after a) 34, b) 364 and c) 728 days of decay during the experiment 8. Peaks identities: C_{xdi} = α,ω-dicarboxylic acid containing x acyl carbon atoms, present as its bis-TMS ester; C_{xdi}■: α,ω-dicarboxylic acid containing x acyl carbon atoms, with one carboxyl group methylated and the other trimethylsilylated (produced during base treatment of the extracted potsherd); $C_{m:n}$ = fatty acid with n carbon atoms and m double-bonds, present as its TMS ester. All other compounds were present as their methyl esters, TMS ethers.

4.5 Degradation processes affecting olive oil during vessel storage (Experiment 11)

An experiment was set-up using unglazed potsherds that were kept in a clean flask containing no mushroom compost in order to study the decay of absorbed olive oil during vessel storage (Section 2.3.1)

4.5.1 Potsherd organic carbon and lipid content

Figure 4.20a shows the changes in the total lipid content of the potsherds during the experiment 11. The potsherd lipid content first decreased from 10.1 to 1.1 mg g^{-1} after 125 days of incubation, then increased to 6.1 after 320 days. This indicates that after *ca.* 100 days of incubation, when the total lipid content is the lowest, most of the lipids exist in the "bound" form, and thus are not extracted on solvent-extraction. After *ca.* 100 days of incubation, the components of the "bound" fraction are released, thereby increasing the lipid content of the solvent-extractable fraction

4.5.2 Solvent-extractable fractions

Figure 4.21 shows the partial GC trace of an olive oil residue incubated for 412 days during experiment 11, compared with fresh olive oil. The degraded residue is very similar to those recovered during incubation under "oxidative" conditions (Section 4.4.1). After incubation, no triacylglycerols were detected, and an unresolved complex mixture eluted towards the end of the run. The earliest part of the chromatogram was dominated by short-chain fatty acids (C_8 and C_9) and α,ω-dicarboxylic acids (ranging from C_4 to C_9, dominated by the C_9 homologue). The long-chain free fatty acids were quite depleted, and their distribution was substantially altered, being dominated by $C_{16:0}$, then $C_{18:0}$, and $C_{18:1}$ was only a minor component. No sterols were detected during this experiment.

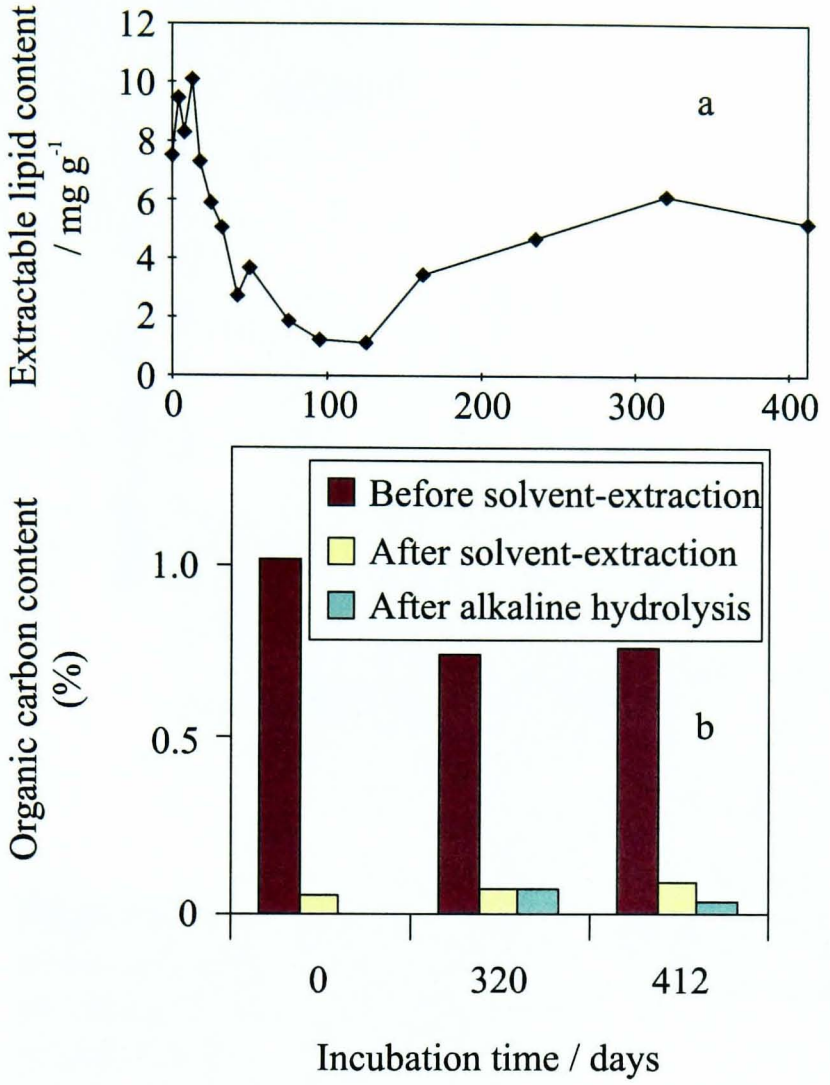


Figure 4.20: a) Lipid and b) organic carbon content during experiment 11 investigating the decay of olive oil under laboratory conditions.

4.5.2.1 Free fatty acids and triacylglycerols abundance

Figure 4.22 shows the changes in the triacylglycerol and free fatty acid relative abundances during experiment 11. The triacylglycerol relative abundance decreased very sharply after 25 days of incubation from 95.6 to 0 % and no triacylglycerols were detected thereafter. The free fatty acid relative abundance remained low (*ca.* 10%) throughout the experiment. These trends are very similar to those that were observed during the experimental decay of olive oil under "oxidative" conditions (Section 4.2.1).

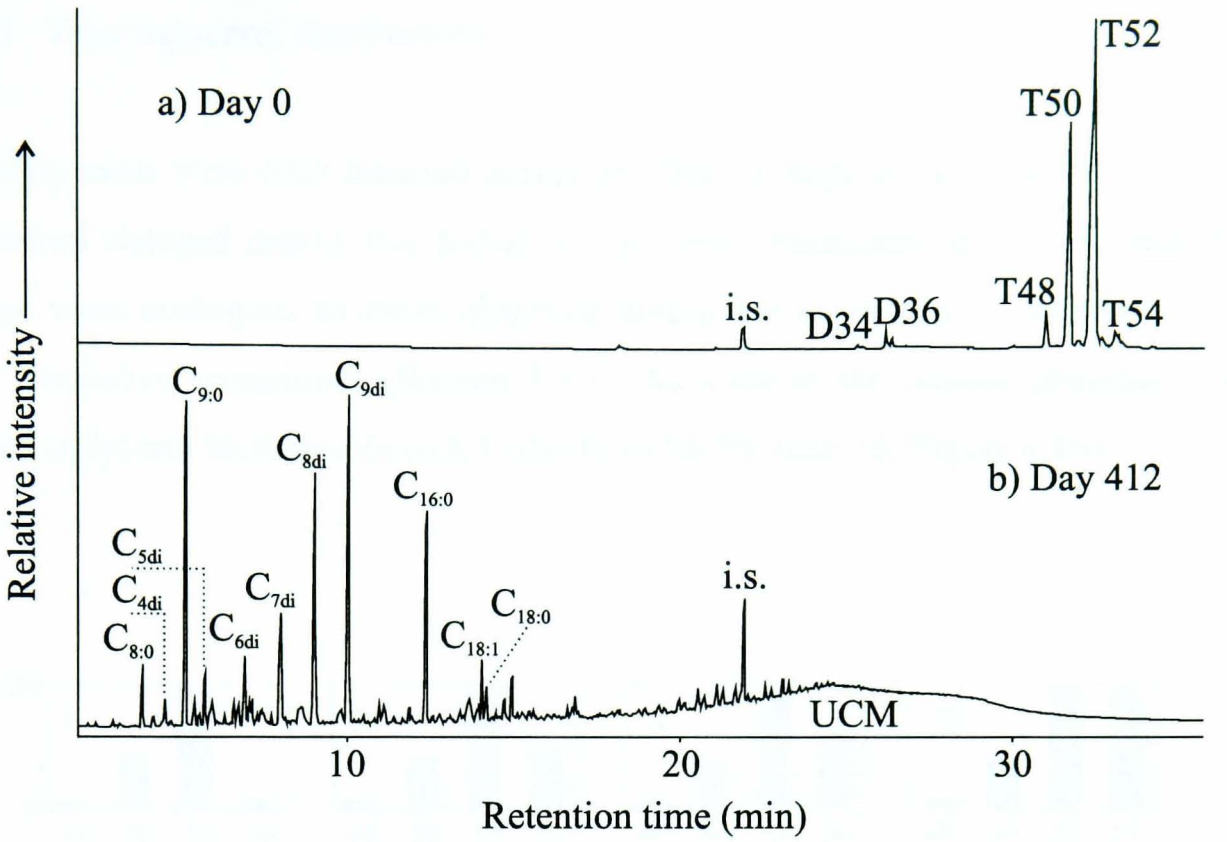


Figure 4.21: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions recovered after a) 0, and b) 412 days of incubation during the experiment 11 investigating the decay of olive oil under laboratory conditions. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; i.s. = internal standard (*n*-tetratriacontane); D34 and D36 = diacylglycerol containing 34 and 36 acyl carbon atoms respectively; T48-T54 = triacylglycerols containing 48 to 54 acyl carbon atoms; UCM = Unresolved Complex Mixture.

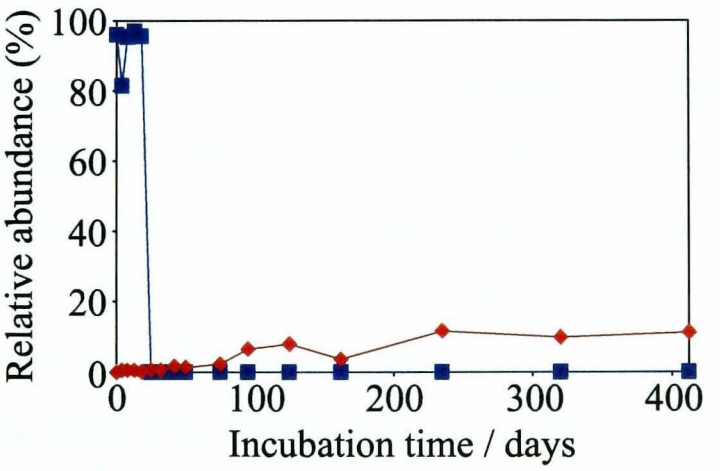


Figure 4.22: Changes in the relative abundance of the triacylglycerols (■) and free fatty acids (◆) during experiment 11 investigating the decay of olive oil under laboratory conditions.

4.5.2.2 Triacylglycerol distributions

Triacylglycerols were only detected during the first 18 days of the experiment 11. Their distribution changed during this period, as the peaks broadened and overlapped. These changes were analogous to those observed during the experimental decay of olive oil under "oxidative" conditions (Section 4.4.1). As a result, the relative abundance of the C_{54} triacylglycerol increased from 4.5 (day 0) to 36.7% (day 18; Figure 4.23).

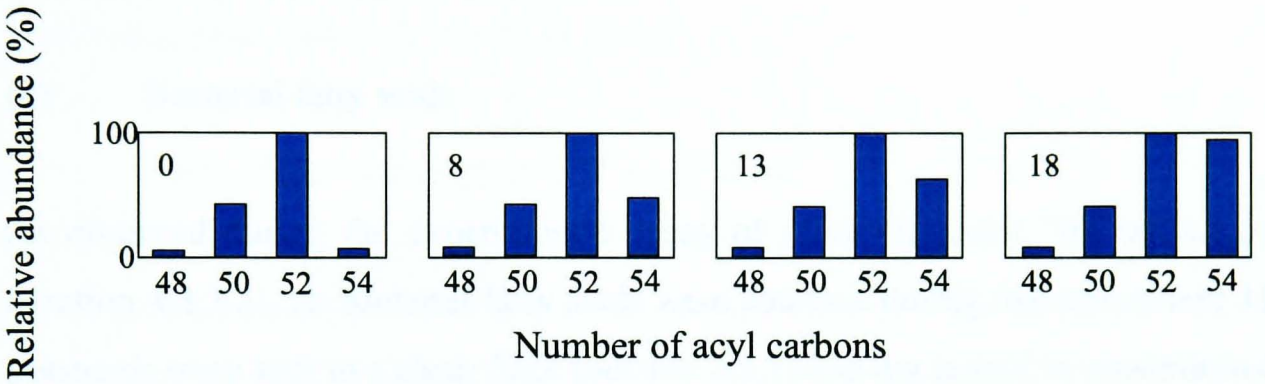


Figure 4.23: Triacylglycerol distributions after 0, 8, 13 and 18 days of incubation during experiment 11.

4.5.2.3 Fatty acids

(i) Free fatty acid ratios

The free fatty acid distribution in the residues obtained during experiment 11 was substantially altered compared to the fatty acid distribution of the fresh olive oil used in the experiment. Figure 4.24 shows the changes in the $C_{18:1}/C_{16:0}$ and $C_{18:0}/C_{16:0}$ ratios during this experiment. The $C_{18:0}/C_{16:0}$ ratio remained constant throughout the experiment, and close to the value for the fresh olive oil used in the experiment. In contrast, the $C_{18:1}/C_{16:0}$ ratio was significantly lower than that of the fresh oil, and decreased rapidly during the experiment, as the unsaturated $C_{18:1}$ was reduced in abundance. Similar trends were observed during the experimental decay of olive oil under "oxidative" conditions (Section 4.4.1.2).

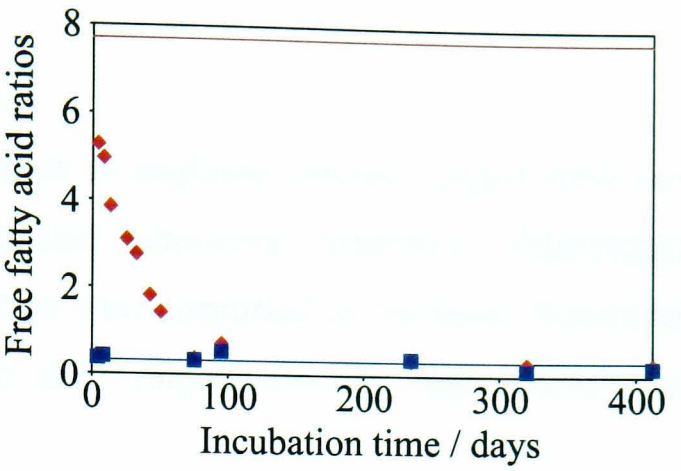


Figure 4.24: Changes in the $C_{18:1}/C_{16:0}$ (◆) and $C_{18:0}/C_{16:0}$ (■) ratios during the experiment 11, compared with the $C_{18:1}/C_{16:0}$ (—) and $C_{18:0}/C_{16:0}$ (—) ratios in the commercial olive oil used as a substrate.

(ii) Bacterial fatty acids

As observed during the experimental decay of olive oil under "oxidative" conditions (Section 4.4.1.2), no bacterial fatty acids were detected during the experiment 11. As the potsherds were kept in a clean flask (Section 2.3.1) and not buried in mushroom compost, it is understandable that bacterial growth was very limited during this experiment.

4.5.2.4 Oxidation products

The production of oxidation products was very significant during experiment 11. Figure 4.25 shows that oxidation products, such as those described in Section 4.5.2, accounted for up to 75.1% of the residue after 320 days of incubation. This is another sign of the importance of unsaturated lipid oxidation during experiment 11.

4.5.2.5 "Bound" fractions

Base treatment of the extracted potsherd released a proportion of the organic carbon that remained in the ceramic after solvent-extraction (Section 4.5.1). The composition of the "bound" fraction recovered after 180 and 320 days of incubation is shown in Table 4.9. This "bound" fraction was dominated by the $C_{16:0}$ and $C_{18:0}$ fatty acids. The $C_{18:1}$ was not detectable in the residues which also contained 2 α,ω-dicarboxylic acids (C_8 and C_9), and 2 hydroxy acids, 9-hydroxydecanoic acid and 9,10-dihydroxyoctadecanoic acid. As was observed during the experiment 8 (Section 4.4.2), the relative abundance of the α,ω-

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4.6 Conclusions

Olive oil residues absorbed in unglazed ceramic vessels were readily degraded during incubation under controlled laboratory conditions. Triacylglycerol hydrolysis and unsaturated lipid oxidation were identified as the main degradative reactions affecting olive oil residues, and their importance was highly dependent on the incubation conditions.

The following observations can be made regarding the effects of these two degradative reactions on absorbed olive oil residues, and their effects on other oils and fats will be described in more details in Chapters 7 and 8.

(i) Degradation under "hydrolytic" conditions.

Triacylglycerol hydrolysis was predominant in experiments conducted at low (5° C) to intermediate (25° C) temperature, in moist mushroom compost. Modification of the incubation conditions by increasing or decreasing the incubation temperature or the amount of water in the compost all resulted in a reduction of the rate of hydrolysis, which is consistent with degradation being biologically driven (for more details, see Section 7.2.1). However, the contribution of bacterial components in the degraded olive oil residues was minor in all experiments.

Degradation under "hydrolytic" conditions preserved the free fatty acid and triacylglycerol distributions. However, the unsaturated components of olive oil were affected by "oxidative" processes and yielded a range of oxidation products, including hydroxy acids which were present in both the solvent-extractable and "bound" fractions. Base treatment of the extracted ceramics also enabled the recovery of oxidation products that were not present in the solvent-extractable fraction. Overall, the structure of the oxidation products present in degraded olive oil residues was indicative that chemical oxidation of the unsaturated acyl lipids took place to a limited extent during the incubation of olive oil residues under "hydrolytic" conditions (for more details, see Section 7.1.2).

(ii) Degradation under "oxidative" conditions

Unsaturated lipid oxidation was predominant in the experiments conducted at high temperature (55° C) and in dry mushroom compost. It significantly altered the fatty acid and triacylglycerol distribution of the residues as the unsaturated components were preferentially degraded. A wide range of oxidation products, including hydroxy acids and α,ω-dicarboxylic acids, were present in both the solvent-extractable and "bound" fractions, and their structure was consistent with their formation *via* radical oxidation of oleic acid. Oxidation of the unsaturated acyl moieties clearly took place and yielded a highly complex mixture of relatively polar triacylglycerols unresolvable by HTGC. Overall the structure of the oxidation products recovered from either the solvent-extractable and "bound" fractions clearly reflected the structure of the unsaturated fatty acid they were produced from, and it is very likely that study of oxidation products associated with archaeological ceramics would provide information regarding the original presence of unsaturated commodities such as vegetable and fish oil.

Unsaturated lipid oxidation also affected olive oil residues during the experiment replicating vessel storage. As most vessels would have been stored for various period of time between uses, it is very likely that the majority of archaeological residues would have been affected by oxidative processes, independently of their burial environment.

CHAPTER 5: EXPERIMENTAL DECAY OF DAIRY FATS UNDER LABORATORY AND FIELD CONDITIONS

5.1 Introduction and aims of the chapter

5.1.1 Chemical composition of dairy fats

The chemical composition of dairy fats from a wide range of animal has been reported (Breckenridge and Kuksis, 1967; Gunstone *et al.*, 1986; Fontecha *et al.*, 1998). Ethnographic evidence shows that, in Antiquity, man has been using milk from various animals (Podolák, 1984) but, in British prehistory, the animals most used would have been cows, goats and sheep.

The lipid content of milk is relatively low, between 16 g l^{-1} (horse) to 105 g l^{-1} (deer), that of cow milk ranging between 26 and 60 g l^{-1} (Gunstone *et al.*, 1986). Triacylglycerols are the main constituents of milk fats. Their carbon number distributions are characteristically wide (from 26 to 54 acyl carbon atoms) and show 2 maxima, one at C_{38} (cow) to C_{44} (sheep), and the other at C_{50} or C_{52} (Table 5.1; Breckenridge and Kuksis, 1967; Gunstone *et al.*, 1986; Fontecha *et al.*, 1998).

Table 5.1: Triacylglycerol distributions in cow, goat and sheep milk fat (from Breckenridge and Kuksis, 1967; Gunstone *et al.*, 1986; Fontecha *et al.*, 1998).

Number of acyl carbon atoms	Relative abundance (%)		
	Cow	Goat	Sheep
26	0.1 - 0.6	0.3 - 0.5	0.3
28	0.5	0.8 - 1.2	0.8
30	0.7 - 1.0	1.7 - 2.5	1.5
32	1.3 - 2.3	2.6 - 4.0	2.9
34	3.5 – 7.3	2.9 - 6.2	3.9
36	9.4 – 14.4	5.3 - 9.4	5.9
38	13.1 – 15.9	10.7 – 12.1	7.3
40	9.3 – 11.6	12.6 – 12.8	8.4
42	5.7 – 7.0	9.3 - 12.5	10.4
44	3.6 – 6.7	7.8 - 11.6	12.4
46	3.8 – 7.2	5.8 - 8.1	10.2
48	5.6 - 8.4	2.7 - 5.8	7.0
50	8.2 - 11.7	5.9 – 6.4	8.3
52	5.1 – 14.7	4.9 – 12.7	12.3
54	1.0 - 7.0	2.0 – 10.5	8.0

Table 5.2 summarises the fatty acid distribution in milk fat from goat, sheep and cow and shows the presence of characteristic short and medium-chain fatty acids (< 14 acyl carbon atoms), representing up to 20% of the total fatty acids. The major fatty acids are $C_{16:0}$ (22 – 28%) and $C_{18:1}$ (20 – 30%) with $C_{18:0}$ being present in similar relative abundance as $C_{14:0}$. Other minor fatty acids include branched compounds, mainly the odd-numbered *iso* and *anteiso* $C_{15:0}$ and $C_{17:0}$, which are produced by microorganisms in the rumen (Gunstone *et al.*, 1986). The unsaturated fatty acids are also affected by the action of these microorganisms, which results in milk fat containing several $C_{18:1}$ isomers with different double-bond positions and configuration (Table 5.3; Gunstone *et al.*, 1986).

Table 5.2: Fatty acid distributions in milk fat from goat, sheep and cow.

	Relative abundance (%)		
Fatty acid	Goat	Sheep	Cow
$C_{4:0}$	-	-	3.5 – 4.2
$C_{6:0}$	2.9	2.8	2.2 – 2.5
$C_{8:0}$	2.7	2.7	0.7 – 2.3
$C_{10:0}$	8.4	9.0	1.8 – 2.8
$C_{12:0}$	3.3	5.4	2.4 – 3.0
$C_{14:0}$	10.3	11.8	9.0 – 11.2
$C_{16:0}$	24.6	25.4	22.0 – 27.8
$C_{16:1}$	2.2	3.4	1.8 – 3.0
$C_{18:0}$	12.5	9.0	11.6 – 14.3
$C_{18:1}$	28.5	20.0	24.8 – 30.4
$C_{18:2}$	2.2	2.1	1.2 – 2.4
$C_{18:3}$	-	1.4	1.0 – 2.6

Table 5.3: Relative abundance of the different positional and geometrical isomers of $C_{18:1}$ in cows milk (Gunstone *et al.*, 1986).

	Relative abundance (%)	
Double-bond position	*cis* isomer	*trans* isomer
Δ^{6}	-	1.0
Δ^{7}	-	0.8
Δ^{8}	1.7	3.2
Δ^{9}	95.8	10.3
Δ^{10}	Trace	10.6
Δ^{11}	2.5	36.1
Δ^{12}	-	4.1
Δ^{13}	-	10.6
Δ^{14}	-	9.0
Δ^{15}	-	6.8
Δ^{16}	-	7.5

The stable carbon isotope ratios of $C_{16:0}$ and $C_{18:0}$ in dairy fats in animals fed pure C3 diet have been determined by Dudd (1998) and are shown in Table 5.4. The depletion in ^{13}C of the $C_{18:0}$ fatty acid in dairy fats compared to those in adipose fats enables degraded dairy and adipose fats in archaeological ceramics to be distinguished (Dudd and Evershed, 1999; Dudd, 1999).

Table 5.4: $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in adipose and dairy fats from cow and sheep as determined by Dudd (1999).

	$\delta^{13}C$ (‰)			
	Adipose fats		Dairy fats	
Fatty acid	Cow	Sheep	Cow	Sheep
$C_{16:0}$	-29.7	-32.2	-29.0	-34.0
$C_{18:0}$	-29.1	-31.0	-29.6	-34.0

Cholesterol represents 90% of all the sterols in milk fat, and is mainly present as the free sterol (only 10% is present as cholesterol esters; Gunstone *et al.*, 1986).

5.1.2 Dairy fats in the archaeological record

The beginning of dairying has been dated to *ca.* 4000 B.C. (Simoons, 1973; Boguchi, 1987) with the help of secondary evidence, such as pictures (Kelly, 1997) and vessels described as "cheese-strainer" (Bogucki, 1987). Ethnographic studies suggest that, in prehistory, not only would milk have been directly consumed, but it would also have been transformed to other products, including butter, yoghurt, cheese and liquor (Podolák, 1984; Crabtree, 1987; Bogucki, 1987, Gouin, 1993), and such activities may well have left traces associated with unglazed ceramic vessels. Furthermore, milk was used to seal porous vessels (Cheape, 1988; Bringéus, 1988; Figure 5.1).

Early work on the identification of degraded dairy fats associated with unglazed vessels proceeded *via* saponification of the solvent-extractable fraction to yield fatty acids which were analysed by GC and whose distribution was compared with that found in intact dairy fats (Rottlander and Schlichtherle, 1979; Bourgeois and Gouin, 1995). However, the incidence of dairy products residues based on fatty acid distributions is unexpectedly low [*ca.* 1% of residues analysed in Bristol (Dudd, Berstan and Evershed, personal communication)]. Dudd and co-workers demonstrated that the low molecular weight triacylglycerols characteristic of dairy fats are more susceptible to ester hydrolysis than their longer-chain counterparts, and once released, the short and medium-chain fatty

Figure 5.1: Experimental ceramic vessel in which milk has been boiled for 1h. Note the white waterproof layer on the walls.

acids are not preserved to the same extent as their longer-chain counterparts (Dudd *et al.*, 1998; Dudd and Evershed, 1998, Dudd, 1999). As a result, degraded dairy fats lack these characteristic short-chain components and resemble adipose fats, thereby precluding their identification by simple fatty acid fingerprinting.

The application of GC-C-IRMS to the determination of $\delta^{13}C$ values of fatty acids in degraded residues associated with unglazed vessels has provided a very useful tool for the reliable identification of degraded dairy fats. Dudd and coworkers have determined the stable carbon isotope ratios of individual fatty acids for a range of adipose and dairy fats, and have demonstrated that there are significant differences in $\delta^{13}C$ values between adipose and dairy fats due to the different biosynthetic origins of the $C_{18:0}$ component of these two classes of fats (Dudd and Evershed, 1998; Dudd, 1999). This new criteria was then successfully applied to several vessel assemblages to identify degraded dairy fats (Dudd and Evershed, 1998; Dudd, 1999). Identifications of archaeological residues as degraded dairy fats based on stable isotope comparisons are supported by other compositional criteria, including triacylglycerol distributions and $C_{18:1}$ double-bond positions and configurations (Dudd and Evershed, 1998; Dudd, 1999).

5.1.3 Aims of the chapter

As discussed above, the identification of degraded dairy fats associated with unglazed vessels is now being achieved using a combination of chemical criteria. Preliminary work (Dudd *et al.*, 1998; Dudd, 1999) suggested that some of these criteria, including

triacylglycerol distributions, and double-bond position and configuration, are not robust during vessel burial. The aim of this chapter is therefore to submit a range of dairy fats to experimental decay under both field and laboratory conditions in order to:

(i) Investigate the feasibility of experimental burial of dosed potsherds under field conditions.

(ii) Monitor the changes in chemical composition of dairy residues during experimental decay under field and laboratory (oxidative and hydrolytic) conditions.

(iii) Compare the rate and extent of decay of different dairy fats.

5.2 Experimental decay of dairy fats under field conditions (Experiment 20, 21 and 22)

Potsherds were dosed with butter, milk and heat-treated milk (Section 2.2.2.2) and buried in heavy garden soil, just below the root mat (Section 2.3.2) and sampled at the intervals given in Table 2.4.

5.2.1 Experimental decay of butter fat under field conditions (Experiment 21)

5.2.1.1 Potsherd lipid content

Potsherds dosed with butter fat initially contained high quantities of lipid (66 mg g^{-1}; Figure 5.2). The potsherd lipid content decreased very rapidly during the early stages of the experiment, decreasing to only 17 mg g^{-1} after 25 days of burial. The potsherd lipid content did not vary significantly thereafter remaining between 10 and 20 mg g^{-1}.

5.2.1.2 Free fatty acids and triacylglycerols relative abundance

Figure 5.3 shows the changes in the free fatty acid and triacylglycerol relative abundance during the experiment 21. The triacylglycerol relative abundance decreased from 99% (day 0) to 57% (day 386) while the free fatty acid content increased from 0 (day 0) to 39% (day 386). As shown in Table 5.5, there was an almost linear relationship between the relative abundance of free fatty acid and triacylglycerol with time.

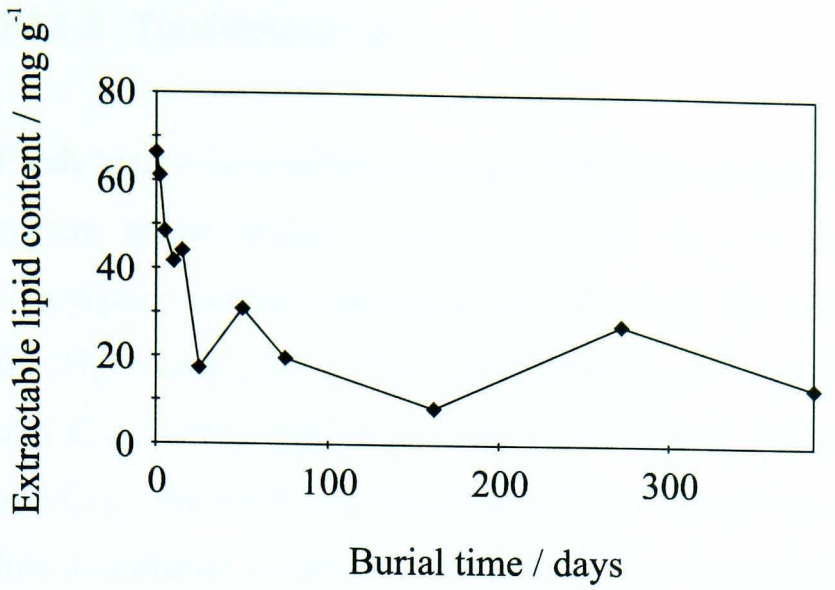

Figure 5.2: Total lipid content of the potsherds during the decay of butter fat under field conditions (Experiment 21).

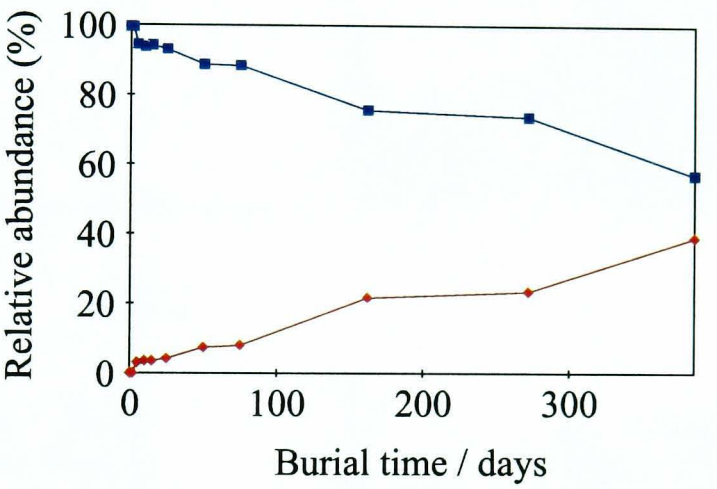

Figure 5.3: Changes in the relative abundance of the free fatty acids (◆) and triacylglycerols (■) during the experimental decay of butter fat under field conditions (Experiment 21).

The changes of the free fatty acid and triacylglycerol relative abundances almost mirrored one another as reflected by the complementarity of the slope of the best-fit lines (-0.099 and 0.094, respectively). Such behaviour is characteristic of a product-reactant relationship, reflecting the exclusive production of free fatty acids from triacylglycerols *via* ester hydrolysis.

Table 5.5: Slope and R^2 values for the linear relationships between the free fatty acid and triacylglycerol abundance, and time.

	Slope	R^2
Free fatty acid	-0.099	0.96
Triacylglycerol	0.094	0.97

5.2.1.3 Total lipid extracts

Fresh butter fat consists mainly of triacylglycerols containing between 26 and 54 acyl carbon atoms (Figure 5.4a). After 162 days of burial under field conditions, these triacylglycerols had undergone partial hydrolysis to yield a low abundance of mono- and diacylglycerols, and mainly free fatty acids. The free fatty acids were dominated by $C_{18:1}$, and $C_{16:0}$. Other significant fatty acids were $C_{18:0}$ and $C_{14:0}$, while the unsaturated $C_{16:1}$ and $C_{14:1}$, the even-numbered $C_{15:0}$ and $C_{17:0}$, and the medium-chain $C_{12:0}$ were present in low abundance. Cholesterol was the only sterol identified in these residues.

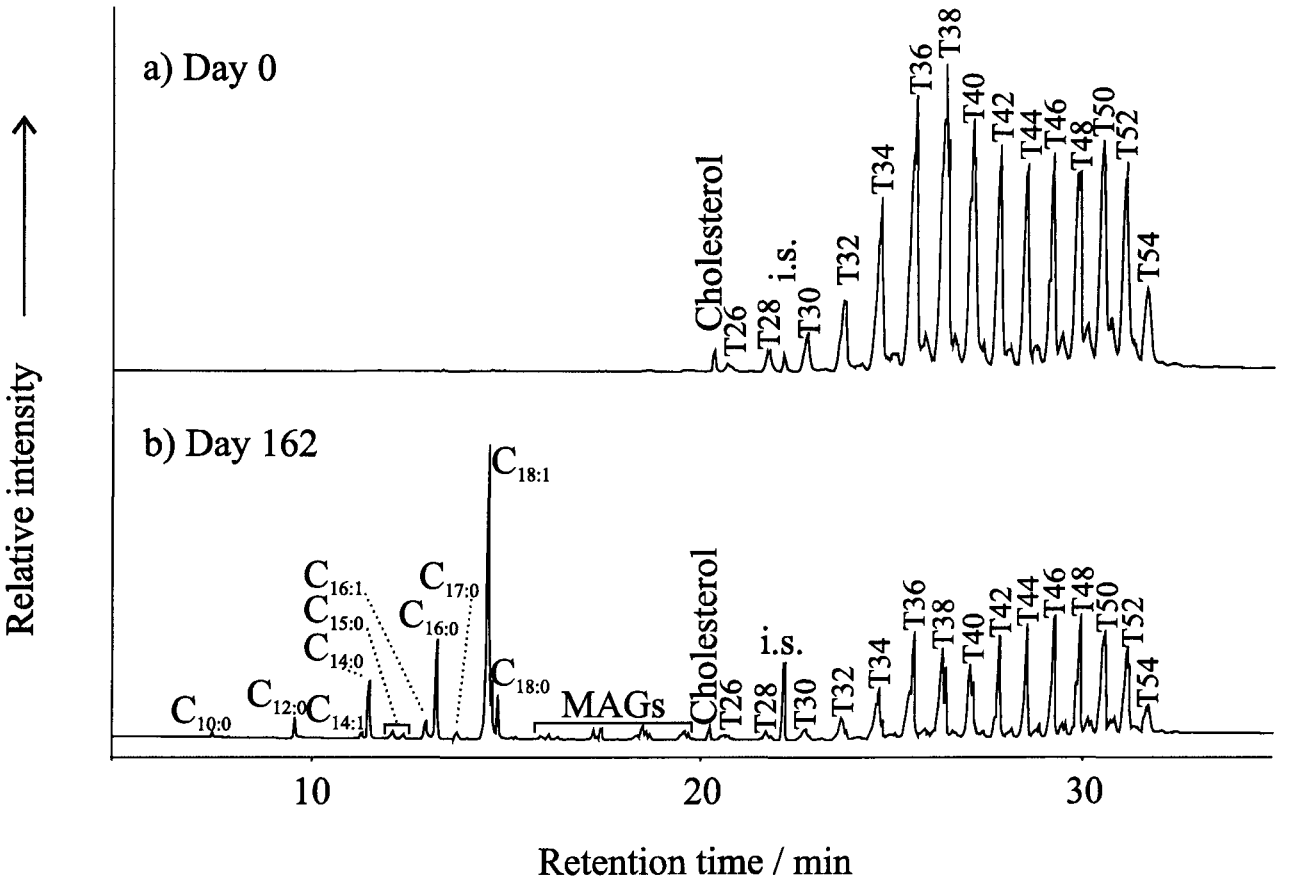

Figure 5.4: Partial HTGC traces of the trimethylsilylated solvent-extractable fractions after a) 0 and b) 162 days of burial during the experimental decay of butter fat under field conditions (Experiment 21). Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; MAGs = monoacylglycerols; i.s. = internal standard (*n*-tetratriacontane); T26-T54 = triacylglycerols containing 26 to 54 acyl carbon atoms.

(i) Fatty acid distribution

During the experimental decay of butter fat (Experiment 21), the free fatty acid distribution was dominated by $C_{18:1}$ (46 – 56%), with $C_{16:0}$ only accounting for 10 – 26% of the free fatty acid fraction (Table 5.6). This was unexpected as the commercial butter used in this experiment contained a significantly higher abundance of $C_{16:0}$ (33%) relative

to $C_{18:1}$ (18%). Interestingly, saponification of the total lipid extract recovered after 272 days of burial yielded a distribution of fatty acids that differed markedly from that of the free fatty acids (Table 5.7).

Table 5.6: Free fatty acid distributions in the solvent-extractable fractions recovered during the experimental decay of butter fat under field conditions (Experiment 21)

	Relative abundance (%)									
Burial time / days	5	10	15	25	50	75	162	272	386	Saponified fresh butter
$C_{12:0}$	1.7	3.5	4.2	2.8	4.3	4.5	2.8	4.6	3.3	3.7
$C_{14:0}$	7.1	8.1	9.0	9.9	10.5	11.5	7.9	8.1	8.9	13.0
$C_{15:0}$	-	1.7	1.9	1.5	1.9	3.2	2.2	2.4	2.7	3.3
$C_{16:1}$	1.8	3.1	3.3	3.0	3.6	3.7	3.7	4.8	4.0	12.7
$C_{16:0}$	26.3	18.8	17.5	19.7	20.4	19.3	14.3	10.4	15.2	33.4
$C_{17:0}$	1.8	0.8	1.0	-	1.0	1.7	1.5	1.5	1.8	2.0
$C_{18:1}$	51.6	51.6	52.7	51.9	45.8	45.8	59.8	59.8	55.8	18.4
$C_{18:0}$	9.7	9.7	7.3	9.7	8.8	8.2	6.2	5.1	7.1	13.4

The total fatty acid distribution of the residues was more similar to that of the commercial butter used in this experiment than that of the free fatty acid fraction released through hydrolysis. The high abundance of $C_{18:1}$ in the free fatty acids could be explained by the fact that (i) this fatty acid is widespread in bacteria and could therefore originate from bacterial contamination (Zellus, 1999) and (ii) unsaturated fatty acids are usually esterified to the *sn*-3 position of dairy triacylglycerols, which limits steric hindrance and enables rapid hydrolysis (Gunstone *et al.*, 1996).

(ii) Double-bond position and configuration in $C_{18:1}$

The double bond position and configuration in the $C_{18:1}$ fatty acid in fresh butter fat, and in butter fat buried for 386 days were determined and are shown in Figure 5.5. Fresh butter contains a high abundance of oleic acid (*cis*-$C_{18:1}\Delta^{9}$; 77.3%) and of *trans*-$C_{18:1}\Delta^{11}$, (14.0%). Other minor isomers (*cis*-$C_{18:1}\Delta^{11}$, *trans*-$C_{18:1}\Delta^{10}$, *trans*-$C_{18:1}\Delta^{12}$, *trans*-$C_{18:1}\Delta^{13}$, *trans*-$C_{18:1}\Delta^{14}$ and *trans*-$C_{18:1}\Delta^{15}$) accounted for *ca.* 1% of the $C_{18:1}$ fatty acid each. The distribution of the $C_{18:1}$ isomers was very similar indeed to butter fat subject to burial for 386 days.

Table 5.7: Comparison of the free fatty acid and total fatty acid distributions in the total lipid extract recovered after 272 days of burial during the experiment 21.

	Relative abundance (%)	
	Total fatty acids	Free fatty acids
$C_{10:0}$	1.4	1.9
$C_{12:0}$	3.7	4.6
$C_{14:1}$	0.9	1.6
$C_{14:0}$	13.4	8.1
$C_{15:0}$	2.7	2.4
$C_{16:1}$	1.6	4.8
$C_{16:0}$	35.2	10.4
$C_{17:0}$	2.6	1.5
$C_{18:1}$	24.0	59.8
$C_{18:0}$	14.6	5.1

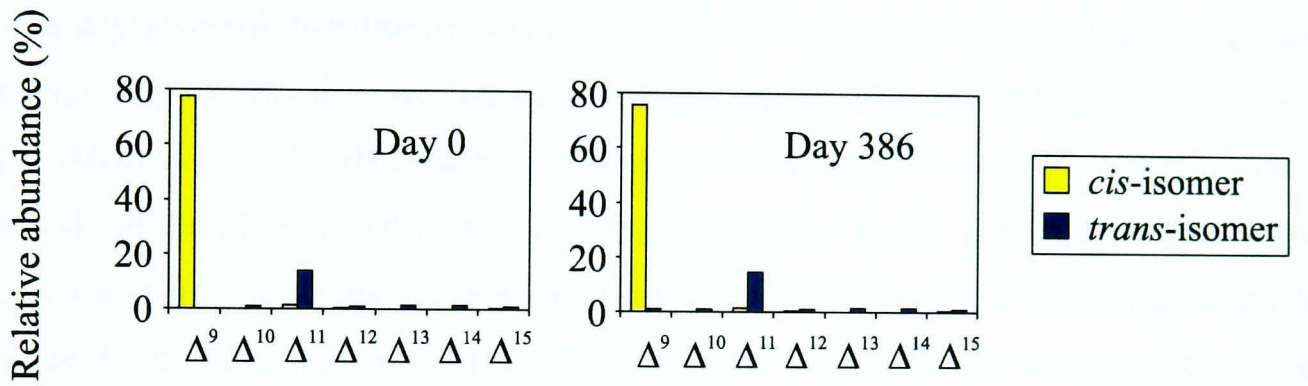

Figure 5.5: Distributions of the isomers of $C_{18:1}$ in fresh butter, and butter buried for 386 days during the experiment 21.

(iii) $\delta^{13}C$ values of individual fatty acids

Figure 5.6 shows the $\delta^{13}C$ values for individual fatty acids in fresh butter fat, and in butter fat buried for 272 days. The stable carbon isotope ratios in fresh butter range between – 25.7‰ and – 34.3‰, $C_{18:0}$ being the most depleted of the fatty acids. Such values have already been reported by Dudd (1999) who used the low value of $C_{18:0}$ to identify degraded dairy fats in archaeological ceramics. During burial, the $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ fatty acids were not significantly affected by degradation. The robustness of the $\delta^{13}C$ values in degraded dairy fat is consistent with previous experiments in which lamb fat was incubated for 1300 days (Dudd, 1999), and during which the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ remained unaffected.

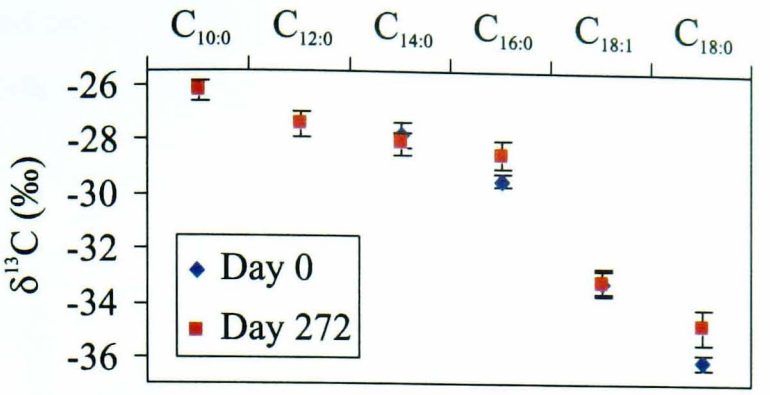

Figure 5.6: $\delta^{13}C$ values of individual fatty acids of fresh butter and of butter residues buried during the experiment 21.

(iv) Triacylglycerol distributions

The triacylglycerol distribution was not altered during the experimental decay of butter fat. Figure 5.7 shows the triacylglycerol distribution of intact butter fat, and of butter fat after 386 days of burial. Both are dominated by low molecular weight triacylglycerols (< 40 acyl carbon atoms), but there were slight increases in the abundance of the higher molecular weight components (> 40 acyl carbon atoms) from 55 to 57% in the degraded residue. In previous work investigating the decay of milk fat under laboratory conditions (Dudd *et al.*, 1998; Dudd, 1999), the low molecular weight triacylglycerol were depleted significantly after incubation. Such losses can be expected to be more important at a later stage of the experiment and result in significant alterations of the triacylglycerol distribution.

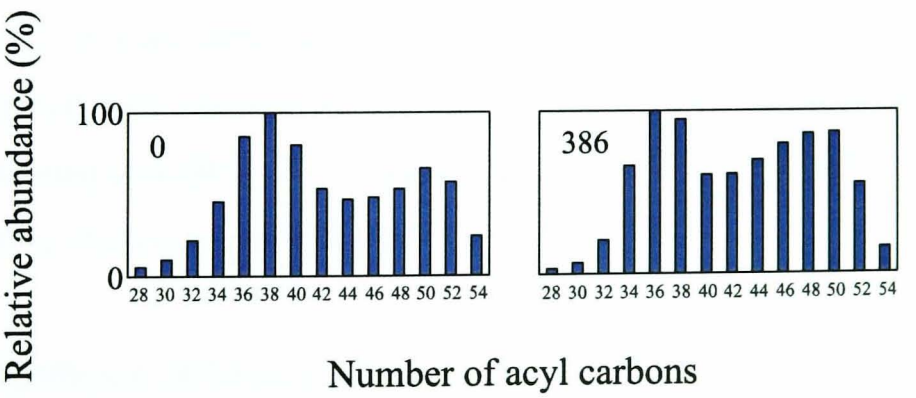

Figure 5.7: Triacylglycerol distributions in residues recovered after 0 and 386 days of decay during the experimental decay of butter fat under field conditions (Experiment 21).

(v) Sterols

Cholesterol was the only sterol identified during the experimental decay of butter fat. Its relative abundance increased from 0.4 (day 0) to 4.7% (day 386), while its absolute

quantity first decreased from 0.2 mg g^{-1} (day 0) and 0.03 mg g^{-1} (day 162), then increased to 0.6 mg g^{-1} (day 386), likely due to cholesterol release from cholesterol esters by hydrolysis.

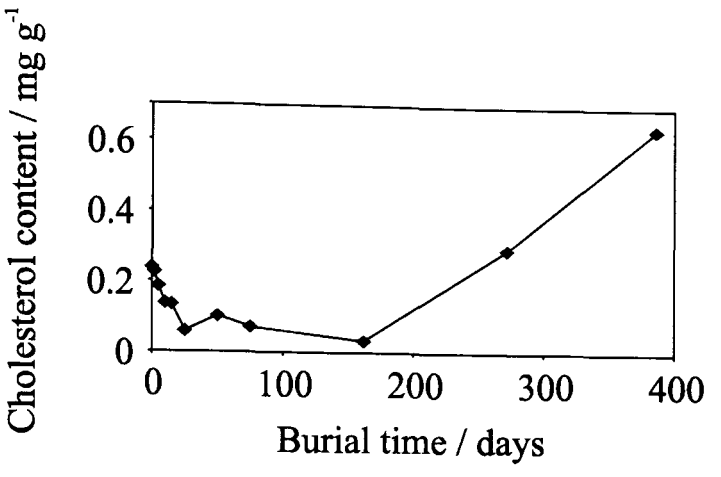

Figure 5.8: Cholesterol content during the experimental decay of butter fat under field conditions (Experiment 21).

5.2.2 Experimental decay of milk fat under field conditions (Experiments 20 and 22)

5.2.2.1 Potsherd lipid and organic carbon content

During the experiment 20, the potsherd total lipid content decreased very rapidly, from 1.3 mg g^{-1} (day 0) to 0.02 mg g^{-1} (day 25) and remained very low (< 5 μg g^{-1}; Figure 5.9a) thereafter, with no organic carbon remaining after solvent-extraction (Figure 5.9b). As decay of milk fat was very fast, another experiment was set-up to investigate the decay of heat-treated milk (Section 2.2.1), in which all natural lipolytic enzymes should have been deactivated (Davídek *et al.*, 1989). Figure 5.10 shows the changes in potsherd lipid content during this experiment (Experiment 22).

There was no significant difference between the experiments investigating the decay of fresh or heat-treated milk. During the experiment 22, the potsherd lipid content decreased very rapidly from 1.4 mg g^{-1} (day 0) to 0.06 mg g^{-1} (day 18) and remained below 0.02 mg g^{-1} thereafter.

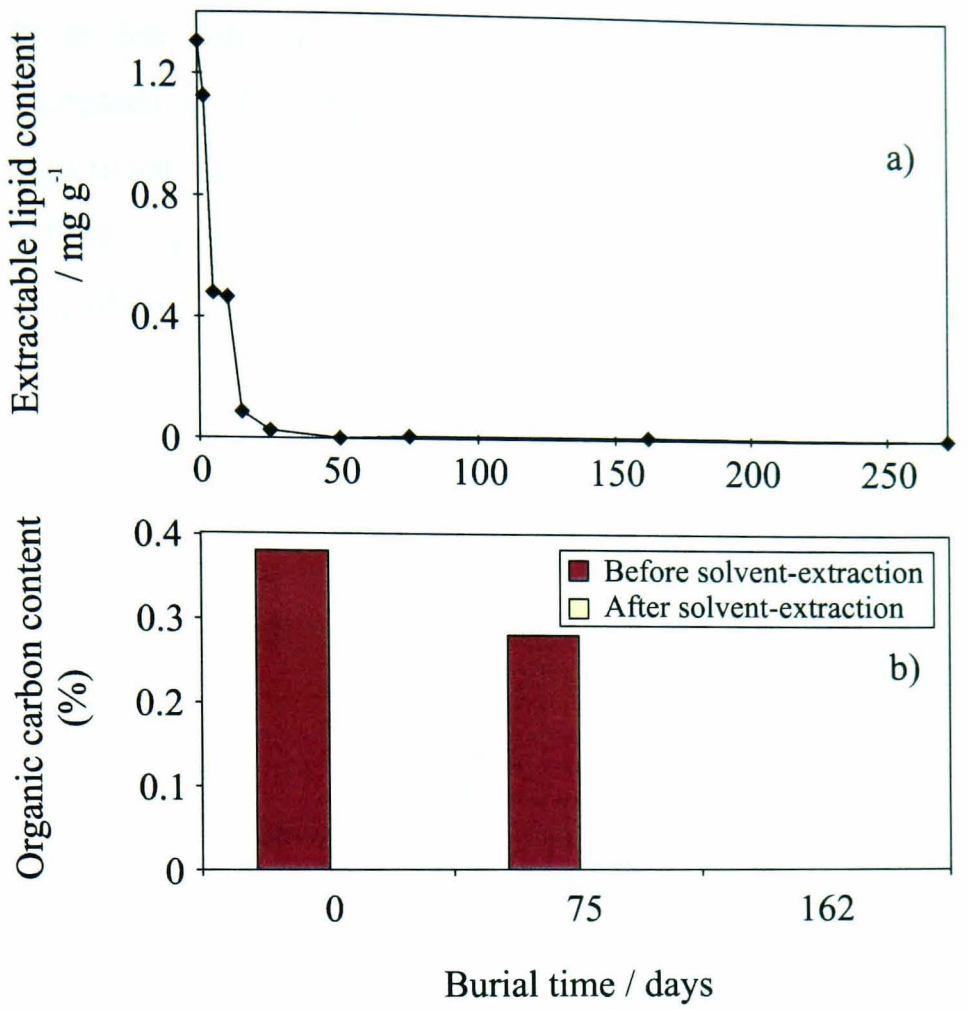

Figure 5.9: Potsherd a) total lipid content and b) organic carbon content during the experimental decay of milk fat under field conditions (Experiment 20).

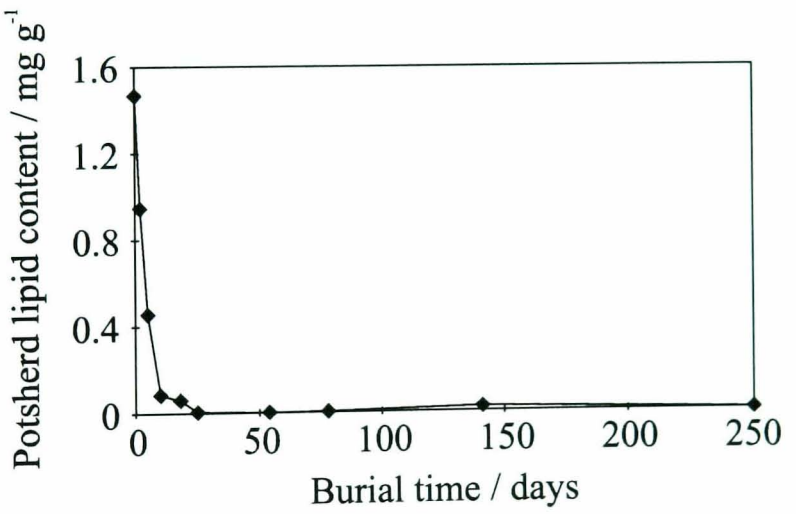

Figure 5.10: Potsherd lipid content during the experimental decay of heat-treated milk under field conditions (Experiment 22).

5.2.2.2 Free fatty acids and triacylglycerols relative abundance

Figure 5.11a shows the changes in free fatty acid and triacylglycerol contents during the experimental decay of fresh milk under field conditions. The triacylglycerol content decreased very rapidly from 1.3 mg g^{-1} (day 0) to 0.02 mg g^{-1} (day 15) and remained very low thereafter. The free fatty acid increased from 0.01 mg g^{-1} (day 0) to 0.4 mg g^{-1} (day

5) as free fatty acids were released from triacylglycerols by ester hydrolysis, then decreased to 0.02 mg g^{-1} (day 25) and remained very low thereafter. There was no significant difference after heat-treatment of the milk used as the substrate (Figure 5.11b), demonstrating that the endogenous milk lipase only plays a limited role in lipid degradation during vessel burial.

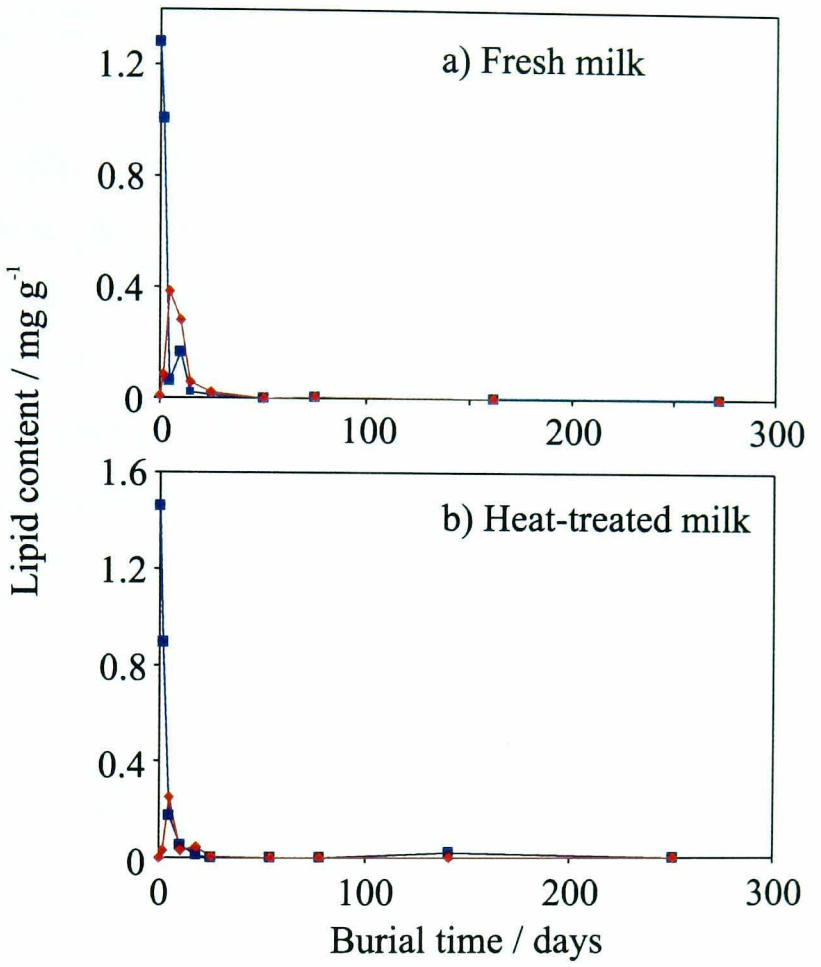

Figure 5.11: Changes in free fatty acid (◆) and triacylglycerol (■) content during the experimental decay of a) fresh milk (Experiment 20), and b) heat-treated milk (Experiment 22), under field conditions.

5.2.2.3 Total lipid extracts

Figure 5.12 shows the partial HTGC traces of residues recovered during the experimental decay of milk fat under field conditions. Fresh milk consists mainly of triacylglycerols containing between 26 and 54 acyl carbon atoms (Figure 5.12a). Free fatty acids are present as minor components and cholesterol is the only sterol. After only 2 days of decay (Figure 5.12b), ester hydrolysis was already evident, releasing minor amounts of mono- and diacylglycerols, and appreciable amounts of free fatty acids. The free fatty acid fraction was dominated by $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$. Other fatty acids included the medium-chain homologues $C_{14:0}$ and $C_{12:0}$ and the branched $C_{15:0}$ and $C_{17:0}$. The triacylglycerols were very depleted after 15 days of burial, and became undetectable after

25 days of decay, at which time the free fatty acids had also become much reduced in abundance.

(i) Fatty acids

During the experimental decay of milk the free fatty acid fraction was dominated by $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ (Table 5.8). The medium-chain fatty acids $C_{12:0}$ and $C_{14:0}$ were much reduced in abundance compared to the fresh milk that was used as a substrate. Both $C_{18:1}$ and $C_{18:0}$ were present in higher quantities than expected, and appeared in different relative proportions to the fresh milk. In the early stages of the experiment $C_{18:1}$ was released in higher proportions than would have been expected from the composition of the fresh milk substrate ($C_{18:1}/C_{18:0}$ = 2.9). As the experiment progresses $C_{18:0}$ was released progressively more, until it appeared in similar abundance to $C_{18:1}$. Preferential hydrolysis of the unsaturated fatty acid $C_{18:1}$ was observed above during the experimental decay of cow butter fat, and the possible explanations for this are given in Section 5.2.1.3.

Table 5.8: Changes in the free fatty acid distributions during the experimental decay of fresh milk under field conditions (Experiment 20).

	Relative abundance (%)							
Burial time / days	2	5	10	15	25	50	75	Saponified fresh milk
$C_{12:0}$	1.2	1.1	0.8	0.7	-	-	-	3.1
$C_{14:0}$	10.3	9.2	3.9	7.4	3.3	4.5	-	18.6
$C_{15:0}$	-	1.9	1.6	1.4	0.5	2.6	-	3.4
$C_{16:1}$	1.3	2.1	1.6	1.7	2.2	3.1	-	1.2
$C_{16:0}$	32.0	30.0	29.8	29.4	29.5	33.5	35.9	31.1
$C_{17:0}$	-	2.0	2.1	2.1	0.3	5.9	-	2.2
$C_{18:1}$	39.8	38.7	37.9	34.6	37.5	27.3	30.9	24.8
$C_{18:0}$	13.9	14.6	22.2	22.8	26.8	23.0	33.2	15.5
$C_{18:1}/C_{18:0}$	2.9	2.7	1.7	1.5	1.4	1.2	0.9	1.6

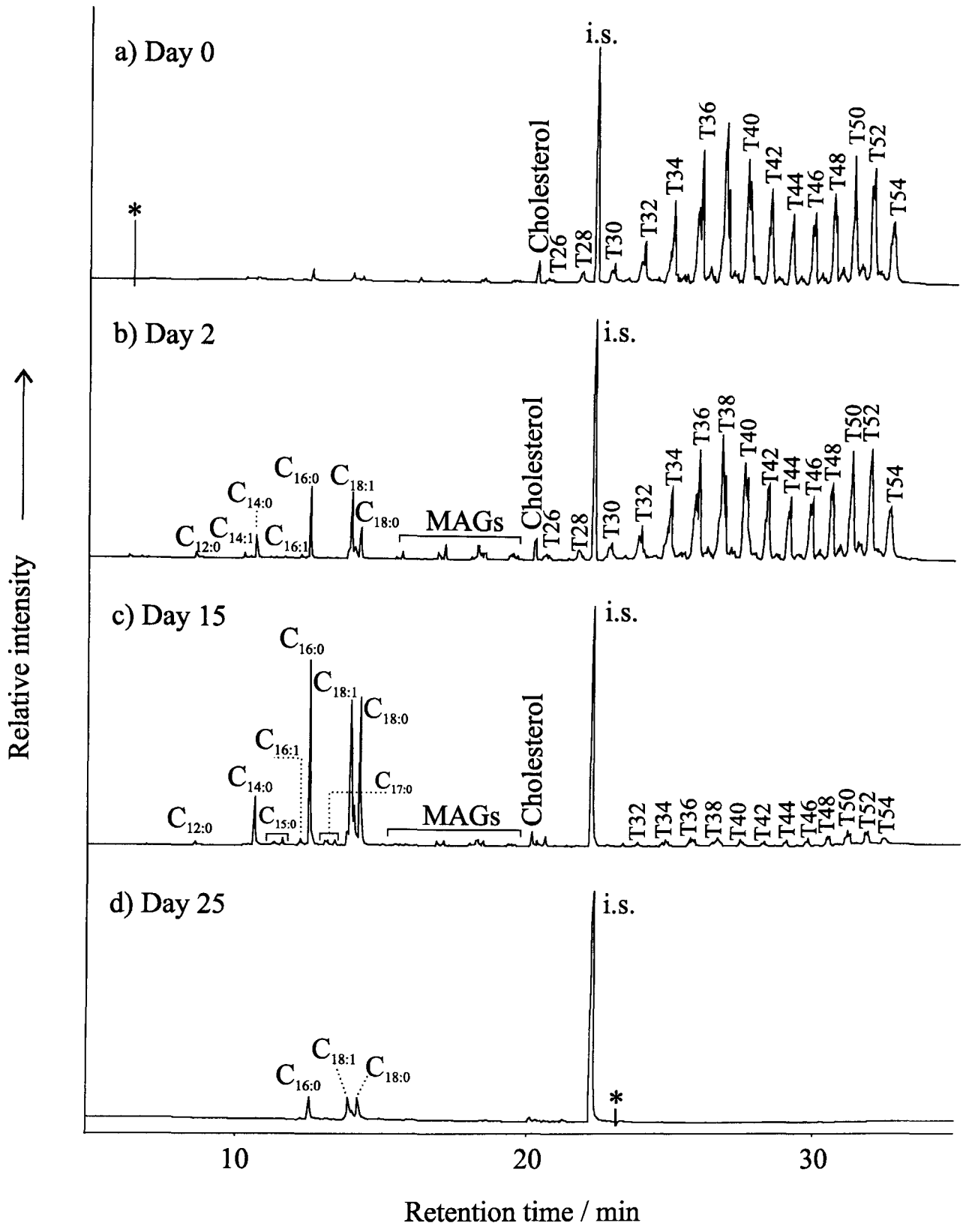

Figure 5.12: Partial HTGC traces of trimethylsilylated solvent-extractable fractions recovered after a) 0, b) 2, c) 15 and d) 25 days of burial during the experimental decay of fresh milk under field conditions (Experiment 20). Peak identities: $C_{m:n}$ = fatty acids with m carbon atoms and n double-bonds; MAGs = monoacylglycerols; i.s. = internal standard (*n*-tetratriacontane); T26-T54 = triacylglycerols containing 26 to 54 acyl carbon atoms; * = electrical spike.

(ii) Triacylglycerol distributions

Before burial (Figure.5.13), the triacylglycerol distribution showed maxima with components containing 38 and 52 acyl carbons, and the profile was dominated by triacylglycerols containing 36 to 40 acyl carbons. After 2 days of decay, the relative

abundance of the triacylglycerols containing less than 36 acyl carbons began to decrease. This pattern continued throughout the experiment, such that after 15 days of decay, the distribution still showed the same 2 carbon number maxima, but with triacylglycerols containing 28 or 30 acyl carbon atoms becoming undetectable, such that the profile was dominated by triacylglycerols containing 50 and 52 acyl carbon atoms. The rapid depletion of triacylglycerols containing short-chain fatty acids have been observed in earlier pilot studies (Dudd *et al.*, 1998; Dudd, 1999), and has been identified as one of the reasons why highly degraded dairy fats become difficult to distinguish from degraded adipose fats (Dudd and Evershed, 1998; Dudd 1999).

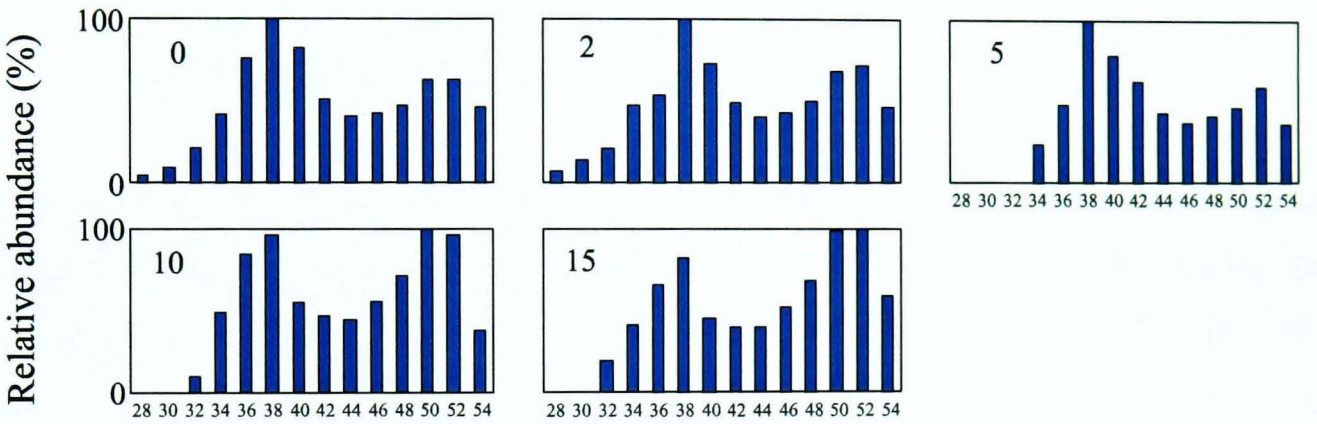

Figure 5.13: Triacylglycerol distributions after 0, 2, 5, 10 and 15 days of burial during the experimental decay of fresh milk fat under field conditions (Experiment 20).

(iii) Sterols

During the experimental decay of milk fat under field conditions, the cholesterol content decreased very rapidly from 8.6 μg g^{-1} (day 0) to 0.2 μg g^{-1} (day 50), and remained very low thereafter.

5.3 Laboratory experiments

5.3.1 Experimental decay of cow butter under laboratory conditions (Experiments 12 and 13)

5.3.1.1 Comparison between "hydrolytic" and "oxidative" conditions (Experiments 12 and 13)

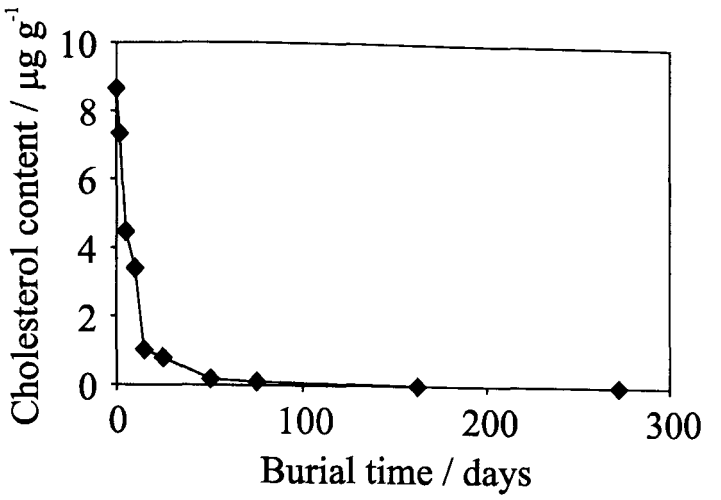

Figure 5.14: Cholesterol content during the experimental decay of fresh milk under field conditions (Experiment 20).

(i) Free fatty acids and triacylglycerols relative abundance

Ester hydrolysis was very slow during the experiments 12 and 13 investigating the decay of cow butter under laboratory conditions (Figure 5.15) as evidenced by the fact that the triacylglycerol relative abundance only decreased from 99.7% (day 0) to 87% (day 349) under "hydrolytic" conditions (Experiment 13; Figure 5.15a), and to 95% under "oxidative" conditions (Experiment 12; Figure 5.15b). In both experiments, the free fatty acid relative abundance increased in similar proportions, suggesting that ester hydrolysis was the dominant reaction during these experiments.

(ii) Potsherd lipid content

The potsherd lipid content recorded during the experiments 12 and 13, shown in Figure 5.16, decreased from 75 mg g^{-1} (day 0) to 12 mg g^{-1} (day 349, "hydrolytic" conditions), and 27 mg g^{-1} (day 349, "oxidative" conditions). The trend in the potsherd lipid content during the two experiments were very similar if allowance is made for differences in the initial lipid content due to the potsherds size (Section 3.2.1.3).

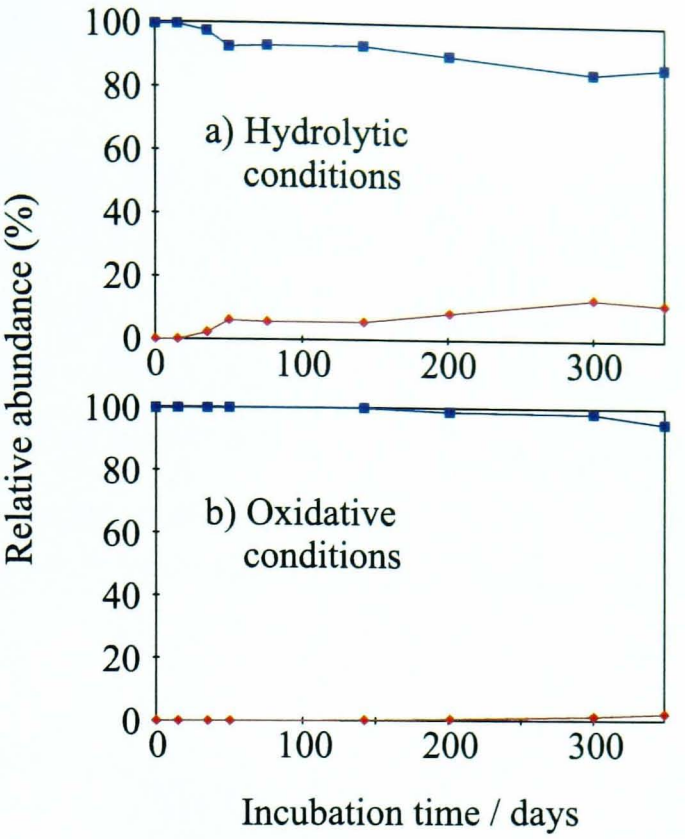

Figure 5.15: Changes in the relative abundance of the free fatty acids (◆) and triacylglycerols (■) during the experimental decay of cow butter fat under a) "hydrolytic" (Experiment 13), and b) "oxidative" conditions (Experiment 12).

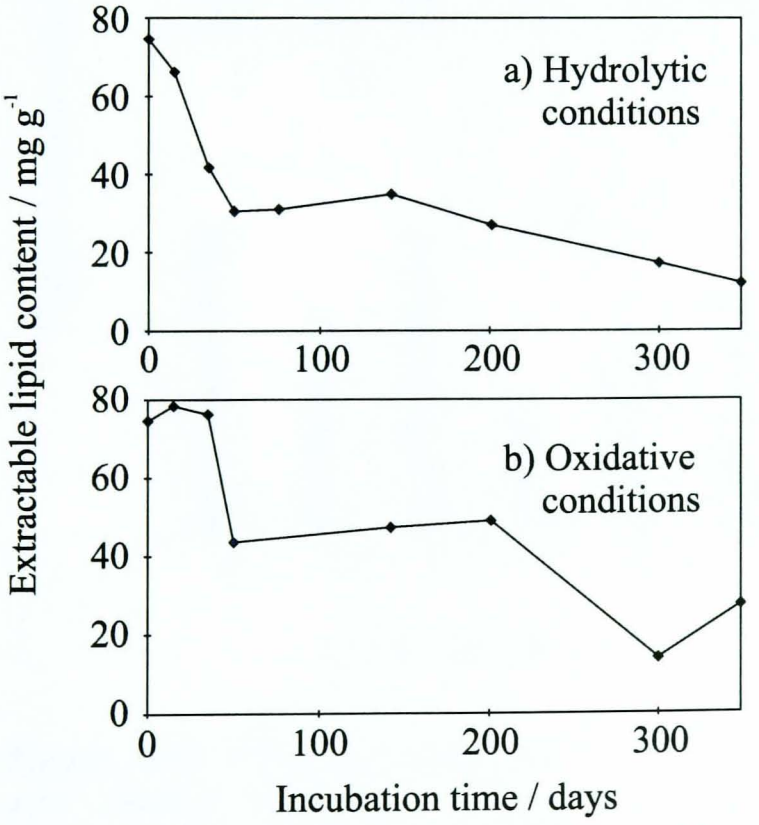

Figure 5.16: Changes in the potsherd lipid content during the experimental decay of cow butter fat under a) "hydrolytic" (Experiment 13), and b) "oxidative" conditions (Experiment 12).

(iii) Potsherd organic carbon content

The potsherd organic carbon content varied significantly during the experimental decay of cow butter (Figure 5.17). They was no significant difference between the experiments 12 and 13, except that, after 349 days of incubation, the potsherds incubated under "hydrolytic" conditions contained less organic carbon than those incubated under "oxidative" conditions (5.1% against 2.5%). This could indicate that, under "hydrolytic" conditions, degradation products are removed from the potsherd, whereas, under "oxidative" conditions, they remain associated to the potsherd, for example as a "bound" fraction.

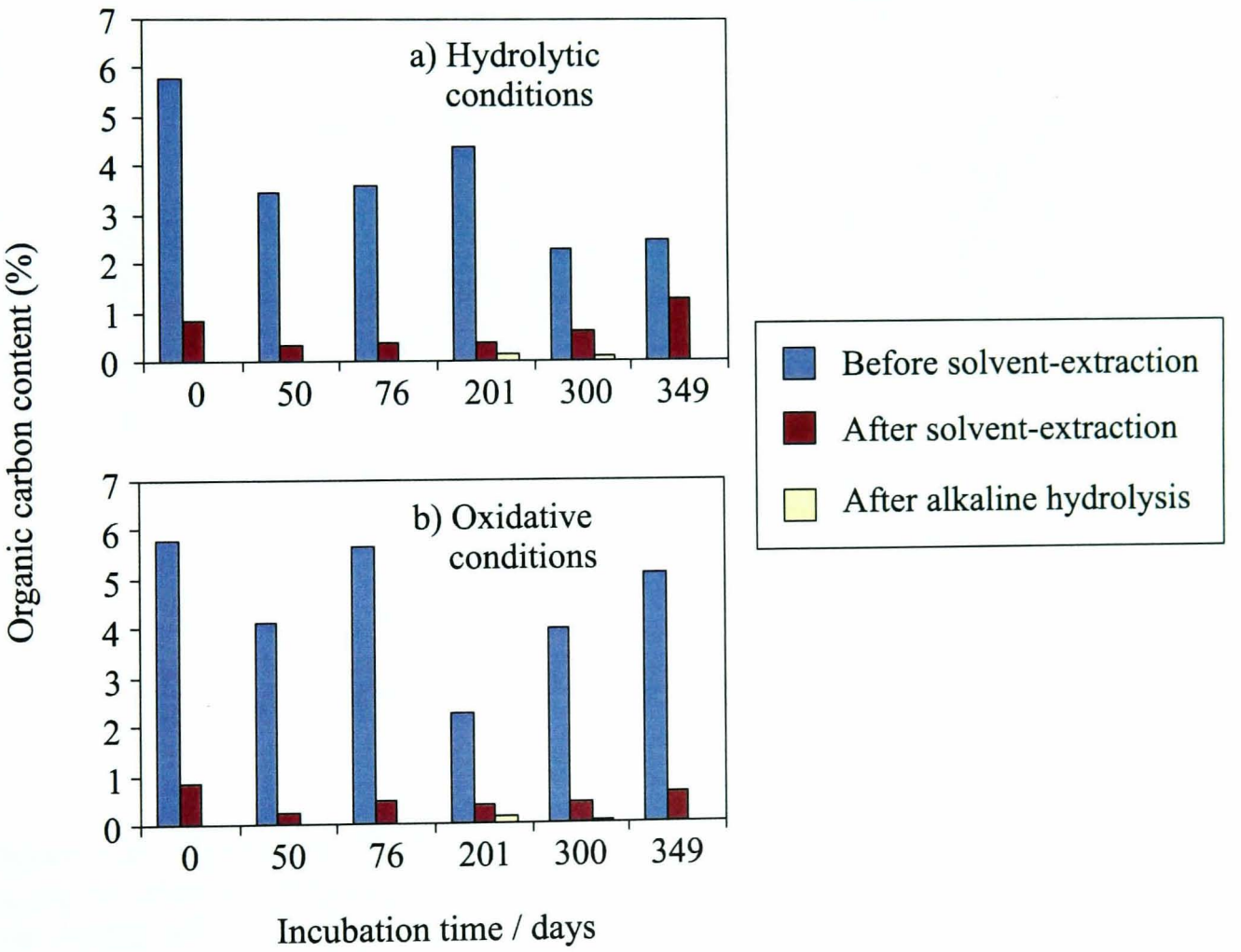

Figure 5.17: Potsherd organic carbon content before and after solvent-extraction, and after alkaline hydrolysis, during the experimental decay of cow butter under a) "hydrolytic" (Experiment 13), and b) "oxidative" (Experiment 12) conditions.

5.3.1.2 Total lipid extracts

(i) "Hydrolytic" conditions

Figure 5.18 shows the partial HTGC traces of degraded cow butter residues recovered after 0, 201 and 349 days of decay under "hydrolytic" conditions (Experiment 13). As was described in Section 5.1.1, intact butter fat consists mainly of triacylglycerols containing 26 to 53 acyl carbon. The only other compound identified was the animal sterol cholesterol.

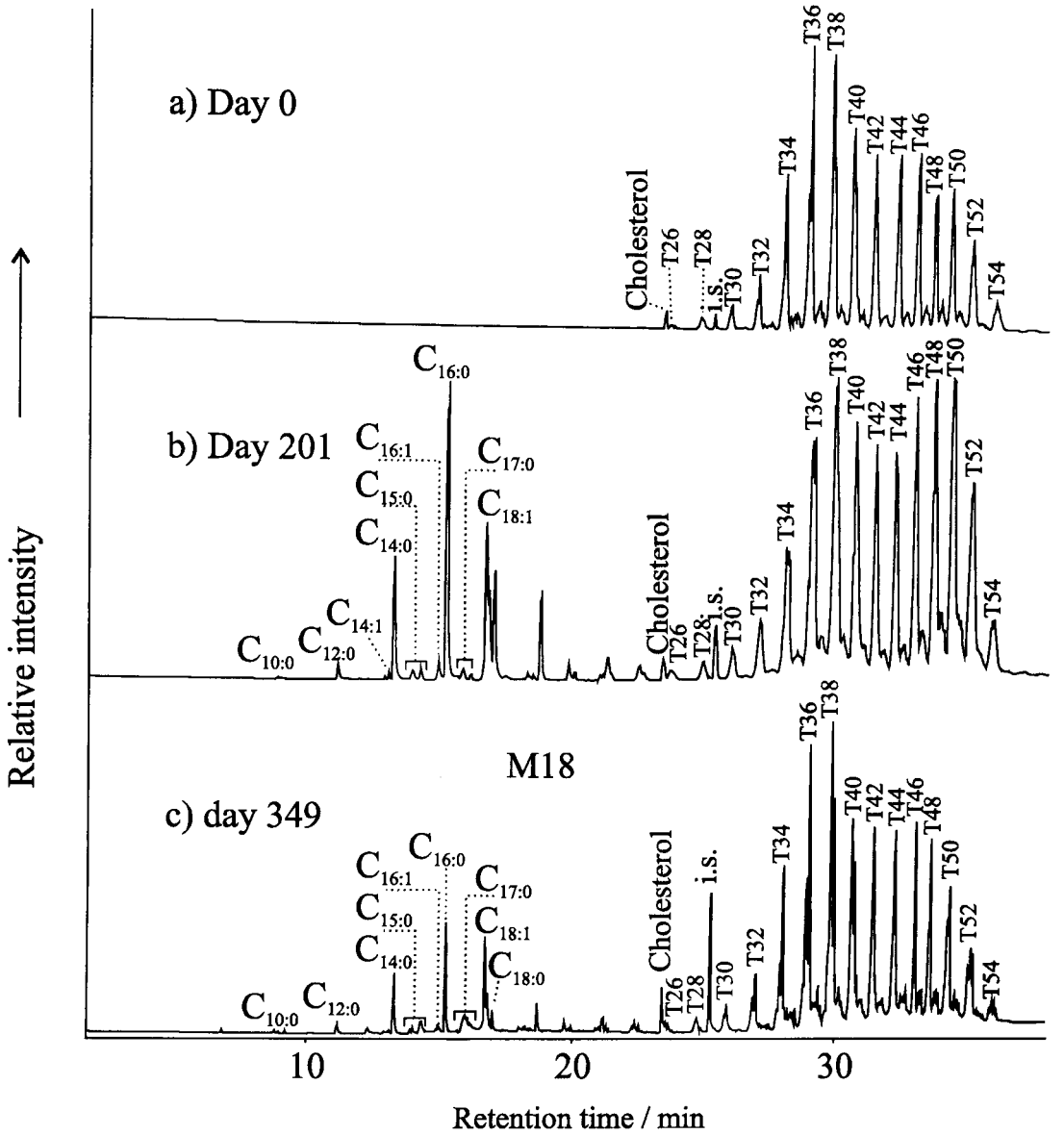

Figure 5.18: Partial HTGC trace of the trymethylsilylated solvent-extractable fractions recovered after a) 0, b) 201, and c) 349 days of incubation during the experiment 13. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; i.s = internal standard (*n*-tetratriacontane); T26-T54 = triacylglycerols containing between 26 and 54 acyl carbon atoms. All compounds were present as their TMS esters and ethers.

After 201 days of decay under hydrolytic conditions (Experiment 13, Figure 5.18b) ester hydrolysis had taken place, yielding abundant free fatty acids from triacylglycerols. The free fatty acid fraction was dominated by $C_{16:0}$ and $C_{18:1}$, and also contained medium-chain fatty acids (with as few as 10 carbon atoms). The free fatty acid fraction of the degraded butter residues contained significantly more $C_{18:1}$ than was expected from the composition of the saponified butter used as the substrate in this experiment (Table 5.9).

The possible explanations for the preferential release of this fatty acid have been discussed in Section 5.2.1.3. The free fatty acid fraction of the degraded residues also contained a lower abundance of medium-chain fatty acids ($C_{10:0}$ and $C_{12:0}$) than expected. This was unexpected as short-chain components are thought to be more susceptible to hydrolysis than their longer-chain counterparts (Dudd, 1999), however, once hydrolysed, they may have been lost from the potsherd by dissolution in the water present in the incubation flasks.

Table 5.9: Free fatty acid distribution in the solvent-extractable fractions recovered during the experiment 13, compared to that in the butter fat used as substrate (after saponification).

Incubation time / day	Relative abundance (%)							
	35	50	76	142	201	300	349	Saponified fresh butter fat
$C_{10:0}$	-	-	-	-	-	0.2	0.5	1.9
$C_{12:0}$	-	1.7	1.3	1.3	2.0	2.1	3.9	3.4
$C_{14:1}$	-	-	-	-	0.5	0.5	0.9	1.0
$C_{14:0}$	7.1	12.0	12.3	10.9	12.3	11.9	12.2	13.2
$C_{16:1}$	-	3.0	3.2	3.4	2.7	3.5	4.1	0.2
$C_{16:0}$	37.3	31.2	31.4	23.9	30.4	27.9	17.0	39.4
$C_{18:1}$	32.3	40.2	39.7	50.7	36.8	42.1	37.9	25.2
$C_{18:0}$	23.3	10.7	10.9	9.8	11.0	9.2	3.1	15.6

The triacylglycerol distribution was altered during the experimental decay of cow butter under "hydrolytic" conditions (Figures 5.18 and 5.19). Fresh cow butter contained triacylglycerols ranging from C_{26} to C_{54}, with 2 maxima at C_{38} and C_{50}. After 201 days of incubation, an increase occurred in the relative abundance of the triacylglycerols containing more than 46 acyl carbons. This trend is similar to that observed during the decay of cow milk and cow butter under field conditions (Experiments 22 and 21, Sections 5.2.2.3 and 5.2.1.3), and that was reported previously during pilot experiments of the decay of milk fat (Dudd *et al.*, 1998; Dudd, 1999).

Cholesterol was the only sterol detected in fresh cow butter. Its concentration in the potsherds is shown (Figure 5.20) to decrease rapidly during the first 50 days of the experiment, from 26 to 9 mg g^{-1}, and remains constant thereafter. This trend is similar to that displayed by the potsherd total lipid content (Section 5.2.1.1).

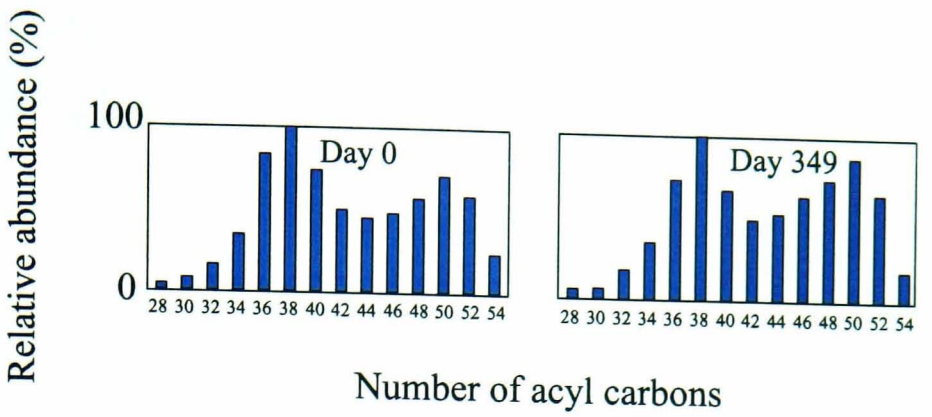

Figure 5.19: Triacylglycerol distributions in fresh cow butter, and cow butter incubated for 210 and 349 days during the experiment 13.

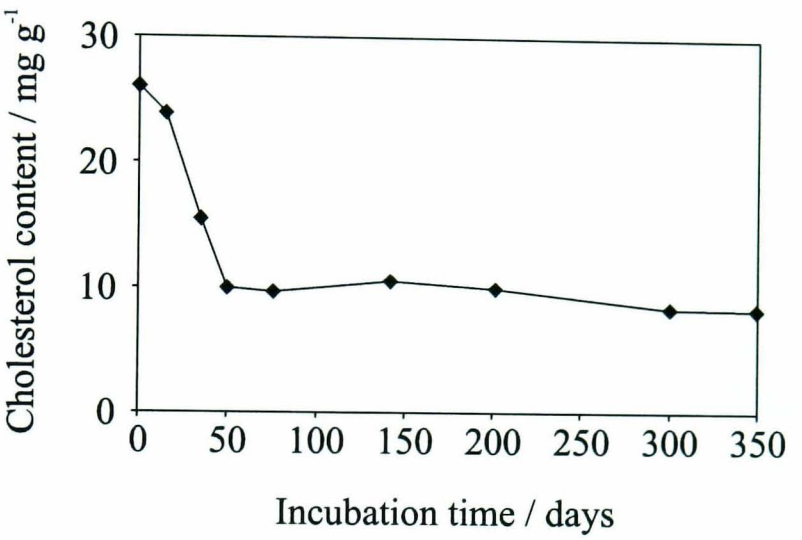

Figure 5.20: Changes in the cholesterol content of the solvent-extractable fraction during the experiment 13 investigating the decay of cow butter under "hydrolytic" conditions.

No oxidation products were detected during the experimental decay of cow butter under "hydrolytic" conditions.

(ii) "Oxidative" conditions

Figure 5.21 shows the partial GC traces of cow butter residues incubated under "oxidative" conditions. After 201 days of incubations, free fatty acids were present; the medium and long-chain $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ were probably produced by ester hydrolysis of the triacylglycerols, whereas the short-chain fatty acids, ranging from $C_{6:0}$ to $C_{9:0}$, and dominated by the $C_{9:0}$ compound, are likely to be oxidation products of the unsaturated fatty acids present in cow butter fat, mainly $C_{18:1}$, which became depleted in the degraded residues compared to the cow butter used as substrate (Table 5.10). The short-chain fatty acids accounted for 2% of the residue after 349 days of incubation.

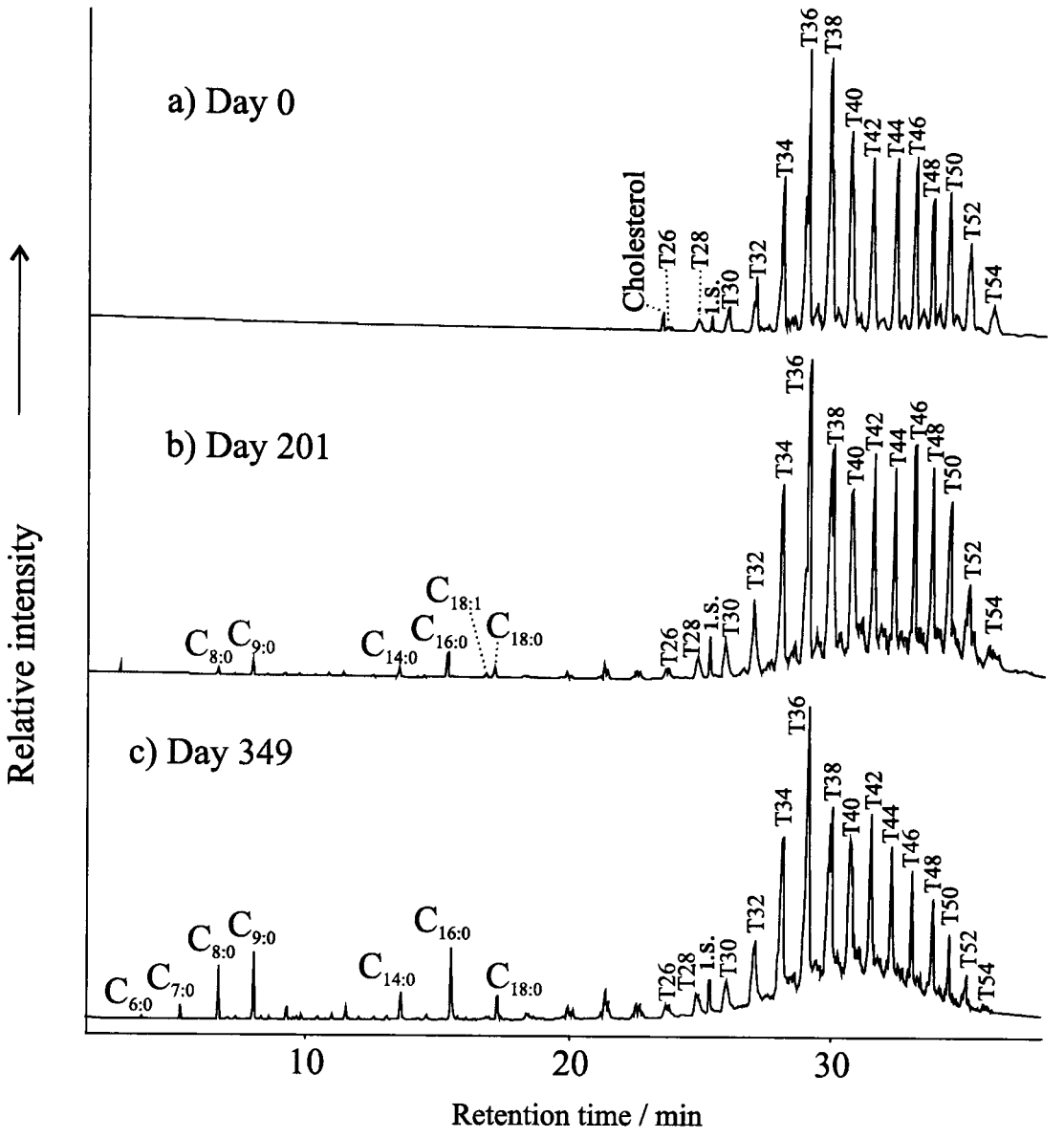

Figure 5.21: Partial HTGC trace of the trimethylsilylated solvent-extractable fractions recovered after a) 0, b) 201, and c) 349 days of incubation during the experiment 12. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; i.s = internal standard (*n*-tetratriacontane); T26-T54 = triacylglycerols containing between 26 and 54 acyl carbon atoms.

The triacylglycerol distribution of the cow butter residues was altered during incubation (Figures 5.21 and 5.22) with the high-molecular weight triacylglycerols (> 44 acyl carbon atoms) being preferentially depleted. This was accompanied by a shift of the lower carbon number maxima from C_{38} to C_{36}. The chromatographic separation between the triacylglycerol peaks decreased with time, likely due to the formation of polar oxidation products (see Section 4.4.1). Overall, the changes of the triacylglycerol distributions are due to (i) the loss of the $C_{18:1}$ acyl moieties from the high-molecular weight triacylglycerols with which it is mainly associated, and, (ii) the formation of polar triacylglycerols containing hydroxy and carboxy groups as a result of oxidation (see Section 4.4.1).

Table 5.10: Free fatty acid distributions in the solvent-extractable residues recovered during the experiment 12, compared to that in the butter fat used as substrate (after saponification).

	Relative abundance (%)			
Incubation time / day	201	300	349	Saponified fresh butter fat
$C_{10:0}$	-	-	4.6	1.9
$C_{12:0}$	-	-	6.4	3.4
$C_{14:1}$	-	-	-	1.0
$C_{14:0}$	11.4	20.9	20.3	13.2
$C_{16:1}$	29.7	-	16.1	0.2
$C_{16:0}$	25.7	59.4	35.4	39.4
$C_{18:1}$	10.4	-	-	25.2
$C_{18:0}$	22.9	19.7	17.1	15.6

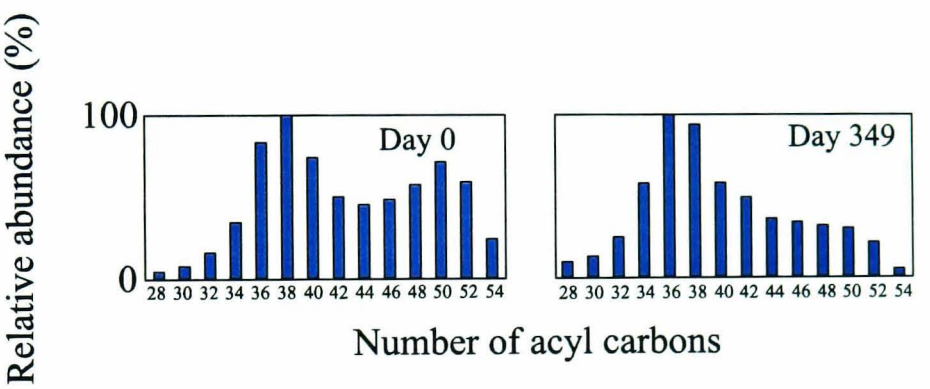

Figure 5.22: Triacylglycerol distribution in fresh cow butter, and in cow butter incubated for 349 days during the experiment 12.

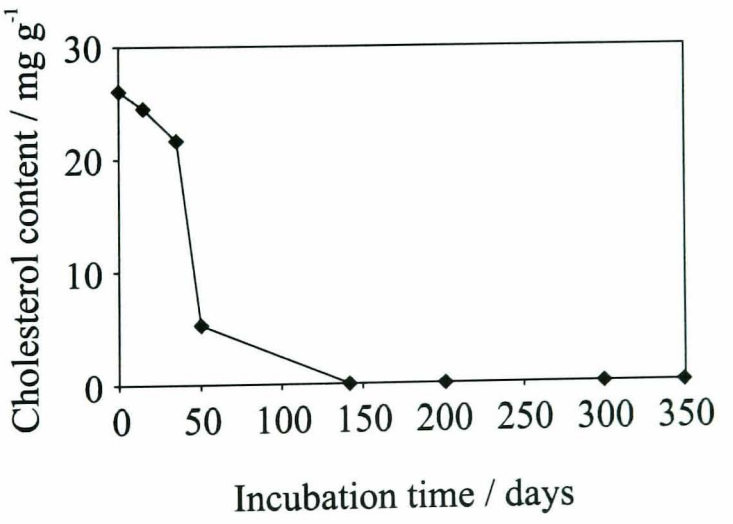

Figure 5.23: Changes in the cholesterol content during the experiment 12 investigating the decay of cow butter under "oxidative" conditions.

Cholesterol was again the only sterol detected in the residues incubated under "oxidative" conditions. Its relative abundance decreased rapidly, from 26 (day 0) to 5.3 (day 50) and 0 mg g^{-1} (day 142 and thereafter).

5.3.1.3 Double-bond position and configuration in $C_{18:1}$

The distribution of the $C_{18:1}$ isomers in the fresh cow butter used in the experiment has been described in Section 5.2.1.3. The $C_{18:1}$ isomer distributions in the degraded residues incubated under "hydrolytic" conditions were very similar to that of the fresh butter fat (Figure 5.24). The residue incubated under "oxidative" conditions, however, shows a reduced abundance of *cis*-$C_{18:1}\Delta^9$ (oleic acid) from 77.3 to 57.2 %, and a corresponding increase in the relative abundance of the other isomers, especially those with a double-bond in the *trans* configuration. The preferential depletion of *cis* configured fatty acids from degraded animal fats has been previously observed in archaeological ceramics (Dudd, 1999), and has been attributed to either to (i) clay-catalysed isomerisation (Stefanova and Disnar, 2000) to yield the more stable *trans* isomers, or (ii) preferential use of one isomer by microorganisms, resulting in the preferential accumulation of the other isomers (Lewis *et al.*, 1999).

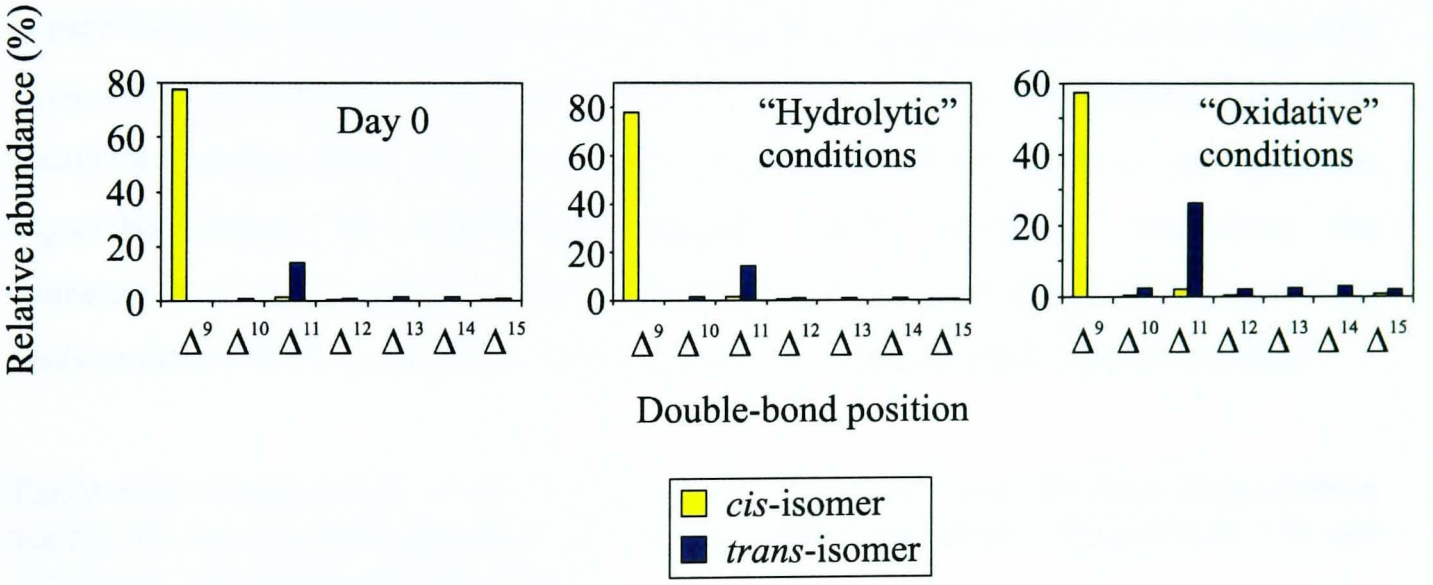

Figure 5.24: Relative abundance of the $C_{18:1}$ isomers in fresh cow butter and cow butter residues following incubation for 349 days under "hydrolytic" (Experiment 13) and "oxidative" conditions (Experiment 12).

5.3.1.4 $\delta^{13}C$ values of individual fatty acids

The stable carbon isotope ratios of individual fatty acids in fresh and degraded cow butter ranged between – 27.6‰ ($C_{14:0}$) to – 34.5‰ ($C_{18:0}$). Similarly to what was seen during the burial of cow butter under field conditions (Section 5.2.1.3), the $\delta^{13}C$ values were not significantly altered during decay (Figure 5.25).

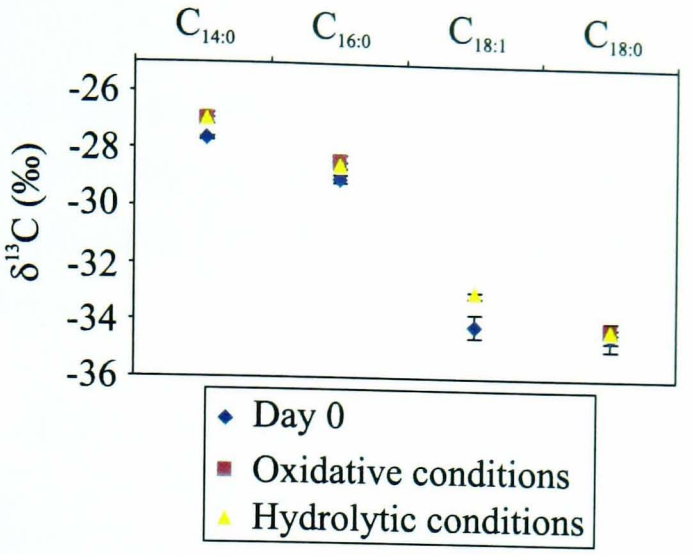

Figure 5.25: $\delta^{13}C$ values of individual fatty acids in cow butter residues incubated under "oxidative" (Experiment 12) and "hydrolytic" conditions (Experiment 13).

5.3.1.5 "Bound" fractions

The composition of the "bound" fractions recovered after base treatment of the extracted ceramics during the experiments 12 and 13 are shown in Table 5.11. During both experiments, the "bound" fraction comprised fatty acids ranging from $C_{12:0}$ to $C_{18:0}$, with $C_{16:0}$ and $C_{18:0}$ dominant. The unsaturated $C_{18:1}$ fatty acid was considerably reduced in abundance compared to the composition of the cow butter used as the substrate, especially during the experiment conducted under "oxidative" conditions; the degradation of $C_{18:1}$ resulted in an increase in the relative abundance of the other fatty acids in the extract. No oxidation products were detectable in these "bound" fractions.

Table 5.11: Composition of the "bound" fraction after 201 and 300 days of incubation during the experimental decay of cow butter under "hydrolytic" (Experiment 13) and "oxidative" (Experiment 12) conditions.

	Relative abundance (%)				
Conditions	"Hydrolytic"		"Oxidative"		
Incubation time / days	201	300	201	300	Saponified fresh butter
$C_{12:0}$	-	0.9	3.3	2.0	3.4
$C_{14:0}$	10.2	11.9	17.5	16.9	13.2
$C_{16:1}$	0.7	-	0.3	-	0.2
$C_{16:0}$	50.5	49.2	51.5	54.3	39.4
$C_{18:1}$	11.7	12.1	2.9	-	25.2
$C_{18:0}$	22.2	21.1	18.8	21.1	15.6

5.3.2 Hydrolytic decay of goat butter (Experiment 14)

5.3.2.1 Potsherd lipid and organic carbon content

During the experimental decay of goat butter, the potsherd lipid content decreased rapidly from 28.5 (day 0) to 1.3 mg g^{-1} (day 201) and remained low thereafter. Likewise, the potsherd organic carbon content decreased from 7.8% at day 0 to 0.2% after 300 days of incubation (Figure 5.26).

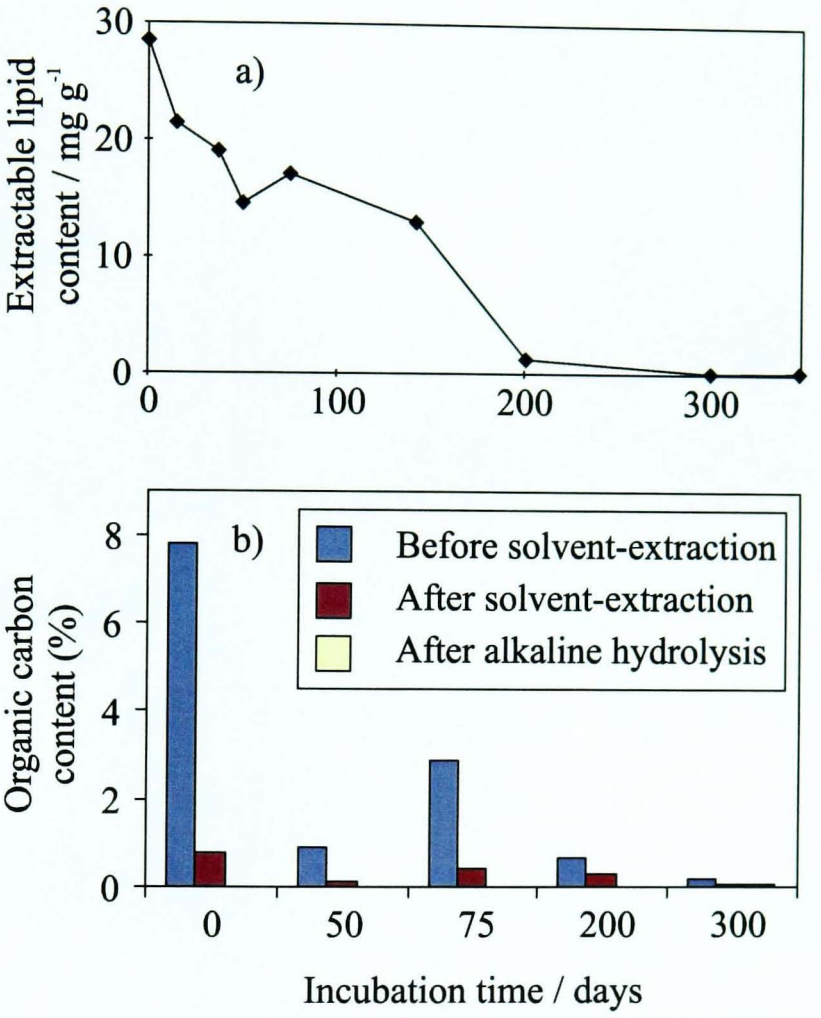

Figure 5.26: a) Lipid and b) organic carbon content of the potsherds recovered during the experimental decay of goat butter under laboratory conditions (Experiment 14).

5.3.2.2 Total lipid extracts

Figure 5.27 shows partial HTGC traces of goat butter residues incubated under "hydrolytic" conditions. Intact goat butter consists mainly of triacylglycerols containing from C_{26} to C_{54} acyl carbon atoms, with a maximum at C_{40}. Cholesterol was the only sterol detected in fresh goat butter. The residues extracted after 37 days of incubation contained free fatty acids, ranging from $C_{10:0}$ to $C_{18:0}$, with high abundance of $C_{16:0}$ and $C_{18:1}$, that were probably released by ester hydrolysis of the triacylglycerols. The triacylglycerol distribution was altered as a result of the preferential loss of the low molecular weight triacylglycerols (containing less than 48 carbon atoms), and now

showed maxima at C_{38} and C_{52}. After 300 days of incubation, triacylglycerols were completely hydrolysed, resulting in a residue consisting exclusively of free fatty acids.

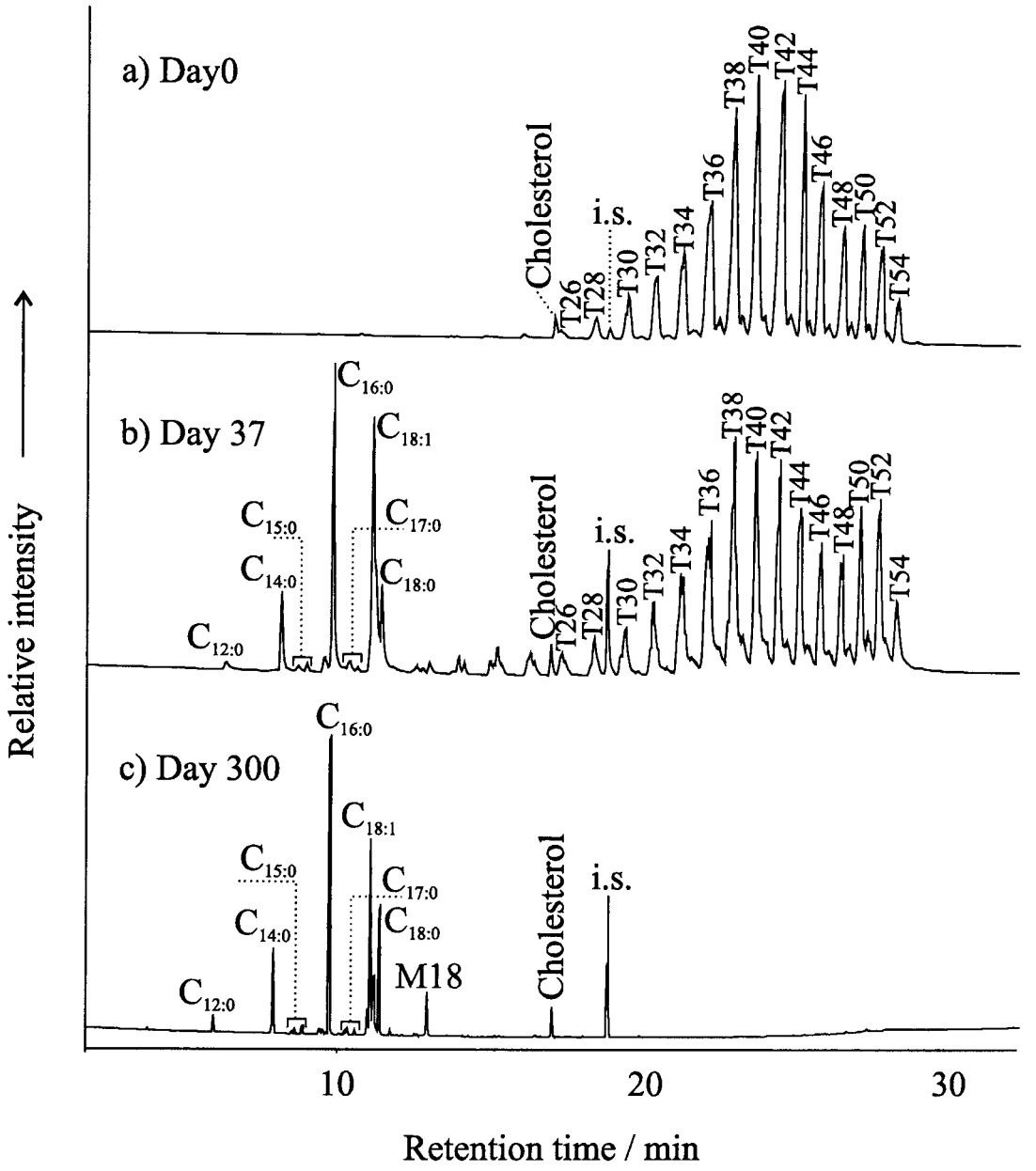

Figure 5.27: Partial HTGC trace of the trimethylsilylated solvent-extractable fractions recovered after a) 0, b) 37, and c) 300 days of incubation during the experimental decay of goat butter (Experiment 14). Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds; i.s = internal standard (*n*-tetratriacontane); T26-T54 = triacylglycerols containing between 26 and 54 acyl carbon atoms.

Figure 5.28 shows the changes of the relative abundances of free fatty acids and triacylglycerols during the experimental decay of goat butter. The triacylglycerol relative abundance decreased almost linearly during the experiment, from 100% (day 0) to 0% (day 300), and there was a corresponding increase in the relative abundance of free fatty acids. Such a trend is consistent with the mechanism of triacylglycerol hydrolysis leading to the release free fatty acids.

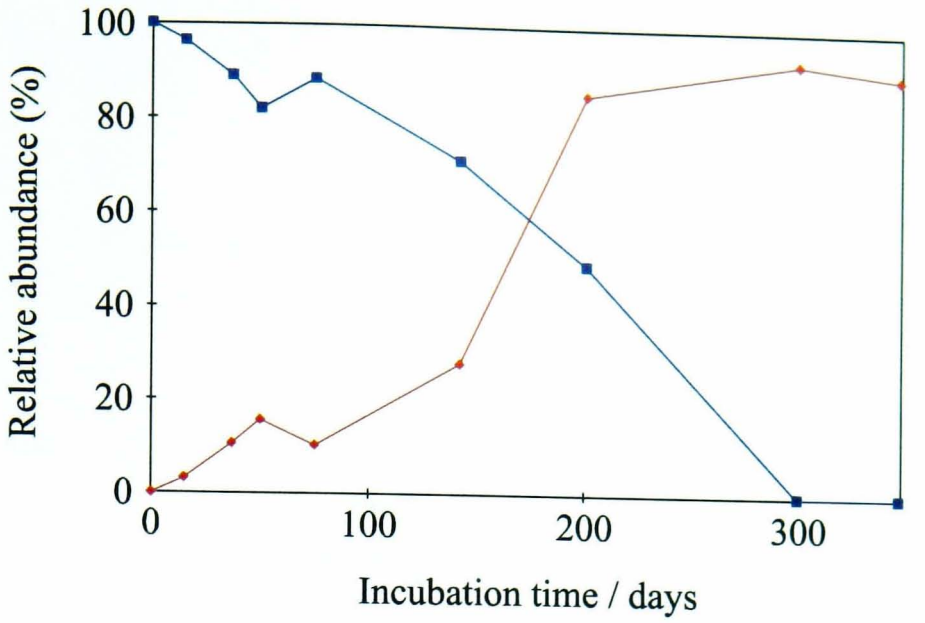

Figure 5.28: Changes in the relative abundances of the triacylglycerols (■) and free fatty acids (◆) during the experimental decay goat butter under "hydrolytic" conditions (Experiment 14).

The free fatty acid distributions of the degraded goat butter residues are shown in Table 5.12. During the first 201 days of incubation, the free fatty distributions were dominated by $C_{18:1}$ which was more abundant than in the fresh goat butter used as the substrate (see Section 5.3.1.2 for possible explanations). After 201 days of incubations, the residues were dominated by $C_{16:0}$, probably because the unsaturated $C_{18:1}$ was depleted by oxidative processes.

Table 5.12: Free fatty acid distributions in the solvent-extractable fractions recovered during the experimental decay of goat butter under "hydrolytic" conditions (Experiment 14).

	Relative abundance (%)								
Incubation time / days	15	37	50	75	142	201	300	348	Saponified goat butter
$C_{12:0}$	-	-	1.5	3.2	2.8	1.3	-	-	3.5
$C_{14:0}$	6.6	8.9	9.9	8.6	6.8	8.0	14.7	13.0	10.6
$C_{15:0}$	-	-	0.2	0.6	1.4	1.7	-	-	1.9
$C_{16:1}$	-	2.0	2.3	2.6	2.6	2.0	-	-	1.2
$C_{16:0}$	37.5	33.9	32.8	22.5	11.1	27.1	57.2	45.6	36.1
$C_{17:0}$	-	-	-	0.5	4.2	1.9	-	-	1.8
$C_{18:1}$	43.4	37.7	42.7	48.9	67.6	46.6	13.8	26.6	30.8
$C_{18:0}$	12.5	17.5	10.1	11.2	2.6	11.4	14.3	14.9	14.2

Cholesterol was the only sterol detected in the degraded goat butter residues, and its content in the potsherd decreased from 17 mg g^{-1} (day 50) to 0.9 mg g^{-1} (day 348).

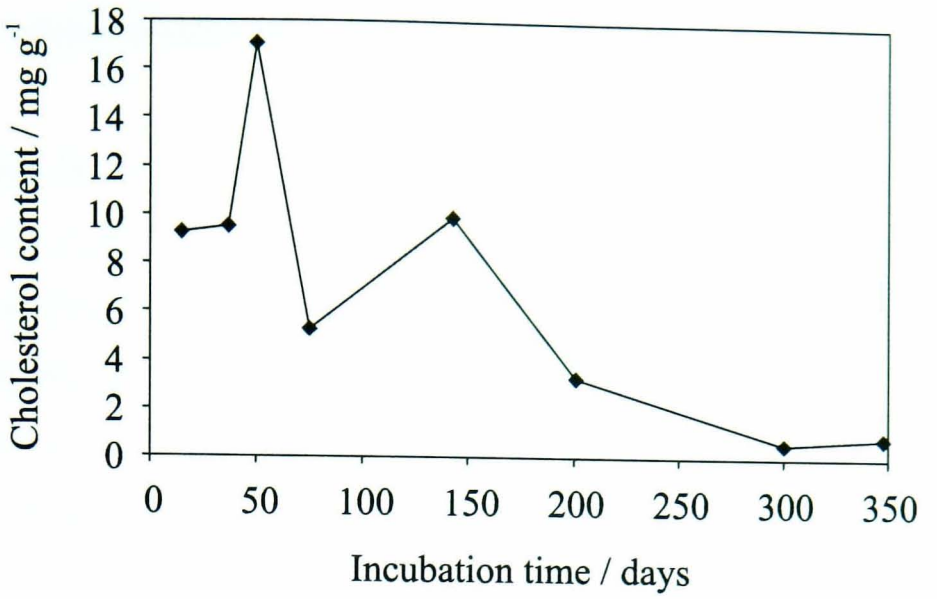

Figure 5.29: Changes in the cholesterol content during the experimental decay of goat butter under "hydrolytic" conditions (Experiment 14).

5.3.2.3 Double-bond position and configuration in $C_{18:1}$

The $C_{18:1}$ isomer distributions in fresh and degraded goat butter are shown in Figure 5.30. Fresh goat butter contained a high abundance of *cis*-$C_{18:1}\Delta^9$ (89%) and minor amounts of *trans*-$C_{18:1}\Delta^{11}$ (7%), *cis*-$C_{18:1}\Delta^{12}$ (1.7%) and *trans*-$C_{18:1}\Delta^{10}$ (1.6%). Degraded goat butter showed a reduce abundance of *cis*-$C_{18:1}\Delta^9$ (62%) and a concomitantly increased abundance of all the other isomers. In addition, the degraded residues contained a mixture of Δ^9 to Δ^{15}-$C_{18:1}$ isomers with the double bond in the *trans* configuration. The distribution of the $C_{18:1}$ isomers in the degraded goat butter residue was similar to that observed in degraded cow butter residues (Section 5.3.1.3) and to that reported in several pottery assemblages (Dudd, 1999, and Sections 3.3.1.2 and 3.3.2.2).

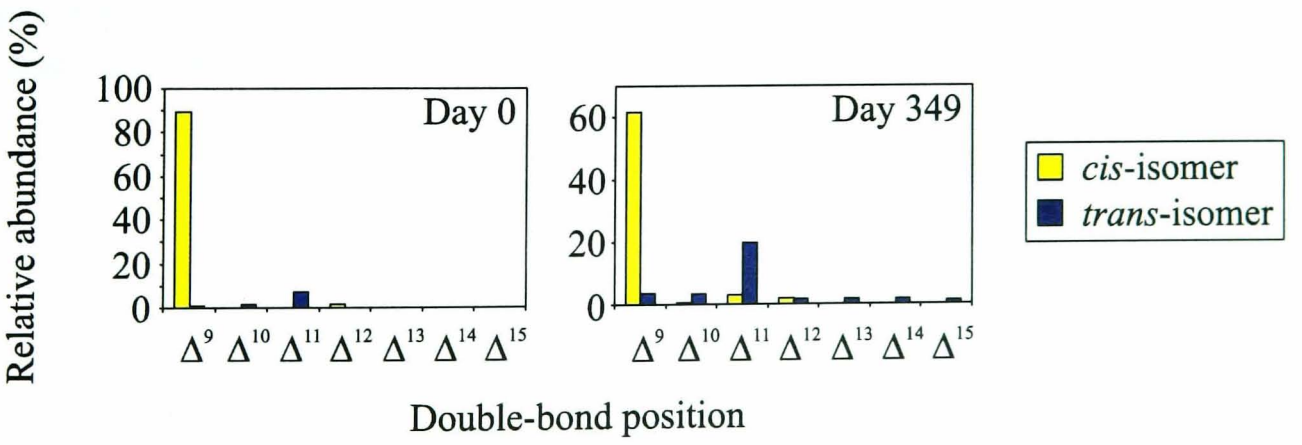

Figure 5.30: Relative abundance of the $C_{18:1}$ isomers in fresh goat butter and goat butter incubated for 349 days during the experiment 14.

5.3.2.4 $\delta^{13}C$ values of individual fatty acids

The $\delta^{13}C$ values of individual fatty acids in fresh goat butter, shown in Figure 5.31, ranged between -25.7‰ ($C_{14:0}$) and –34.3‰ ($C_{18:0}$). After 200 days of incubation, they was a slight enrichment of the $C_{18:1}$ and $C_{18:0}$ fatty acids. A similar enrichment was observed during the experimental decay of cow butter under field (Section 5.2.1.3) and laboratory (Section 5.3.1.4) conditions, and is not considered to be significant as only one sample was analysed.

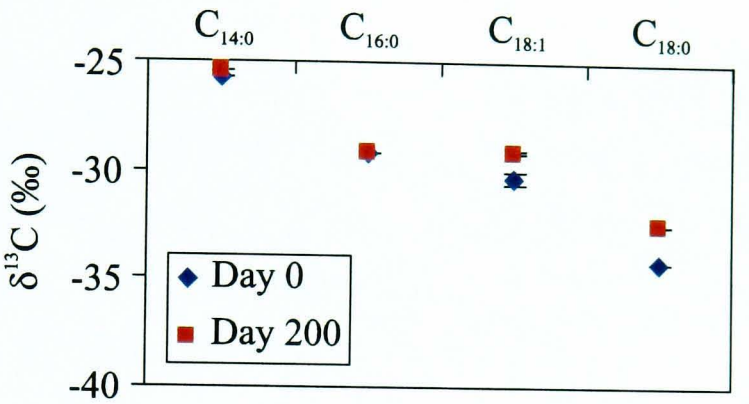

Figure 5.31: $\delta^{13}C$ values of individual fatty acids in intact goat butter and goat butter incubated for 200 days during the experiment 14.

5.3.2.5 "Bound" fractions

The "bound" residue recovered after 300 days of incubation consisted exclusively of the saturated fatty acids $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$. The residue showed a very high abundance of $C_{16:0}$ compared to the goat butter used as the substrate, and did not contain any oxidation products.

Table 5.13: Composition of the "bound" fraction recovered after 300 days of incubation of goat butter during the experiment 14, compared with saponified fresh goat butter.

	Relative abundance (%)	
Incubation time / day	300	Saponified goat butter
$C_{12:0}$	-	3.5
$C_{14:0}$	8.7	10.6
$C_{15:0}$	-	1.9
$C_{16:1}$	-	1.2
$C_{16:0}$	72.9	36.1
$C_{17:0}$	-	1.8
$C_{18:1}$	-	30.8
$C_{18:0}$	18.4	14.2

5.4 Conclusions

The experiments described in this chapter aimed at monitoring the fate of the more labile components of dairy fats during experimental decay under laboratory and field conditions. The effects of "hydrolyic" and "oxidative" decay on dairy residues were found to be similar to those on olive oil residues (Section 4.6), and will be detailed in Chapters 7 and 8, respectively. The main conclusions regarding the effects of decay on dairy residues are:

(i) Triacylglycerol hydrolysis was observed during all experiments, accompanied by overall decrease in potsherd lipid content. The rate and extent of decay was governed by the amount of lipid originally present in the potsherd, as demonstrated by the different rates of degradation of milk fat compared to butter fat, and goat butter fat compared to cow butter fat.

(ii) Degradation of fresh and of heat-treated milk proceeded at the same rate, indicating that decay was driven largely by soil microorganisms, with a negligible influence from lipases naturally present in milk.

(iii) There were very slight enrichments in $\delta^{13}C$ of the $C_{16:0}$ and $C_{18:0}$ fatty acids in all experiments, however, the magnitude of these changes would have little effect on interpretations of archaeological residues.

(iv) The *cis* $C_{18:1}$ isomers were preferentially depleted compared to the *trans* isomers during most experiments.

(v) The "bound" fractions resulting from incubation of dairy fats only contained saturated fatty acids.

(vi) During the experimental decay of dairy fats under "hydrolytic" and field conditions, low molecular weight triacylglycerols (< 40 carbon atoms) were preferentially depleted and the unsaturated $C_{18:1}$ was preferentially released to the free fatty acid fraction. The cholesterol content of the potsherds decreased at the same rate as the total lipid content.

(vii) During the experimental decay of dairy fats under "oxidative" conditions, the of the high molecular weight triacylglycerols (> 48 carbon atoms, i.e. the most unsaturated triacylglycerols), the unsaturated $C_{18:1}$, fatty acid and cholesterol were preferentially reduced in abundance.

Overall, the composition of the dairy residues was significantly altered during all experiments, as the low-molecular weight and the more unsaturated components were preferentially degraded.

CHAPTER 6: EXPERIMENTAL DECAY OF COD LIVER OIL UNDER LABORATORY CONDITIONS

6.1 Introduction and aims of the chapter

6.1.1 Chemical composition of fish oils

Marine oils are characterised by a very high degree of unsaturation (Gunstone *et al.*, 1986) making their analysis very challenging. For instance, marine oils are composed mainly of triacylglycerols containing a high proportion of polyunsaturated long chain acyl moieties which means they are not amenable to high temperature–gas chromatography (Evershed, 1996), resulting in the scarcity of data about the triacylglycerol composition of marine oils.

The fatty acids present in fish oils contain between 14 and 22 acyl carbon atoms, and up to 6 double-bonds (Table 6.1). The main fatty acids are usually $C_{18:1}$ and $C_{16:0}$. Fish oils are characterised by the presence of long-chain polyunsaturated fatty acids such as $C_{20:5}$ [all *cis*-eicosapentenoic acid (EPA)] and $C_{22:6}$ [all *cis*-docosahexaenoic acid (DPA)], whose importance in the diet has recently been recognised (Spector, 1999).

Table 6.1: Fatty acid distributions in trout, cod liver, sardine, herring, anchovy and mackerel (Gunstone *et al.*, 1986; Malainey *et al.*, 1999a; Méndez *et al.*, 1996).

	Relative abundance (%)					
Fatty acid	Trout	Cod liver	Sardine	Herring	Anchovy	Mackerel
$C_{14:0}$	3.4 - 3.7	4.7 – 4.9	6.6 – 7.6	4.6 – 8.4	6.8	7.8
$C_{16:0}$	12.8 - 17.9	11.6 - 12.4	15.5 – 16.2	10.1 – 18.6	22.8	16.0
$C_{18:0}$	3.6 - 4.0	1.8 – 2.1	3.5 – 3.7	0.7 – 2.1	2.7	1.8
$C_{16:1}$	7.0 - 11.2	6.3 - 11.6	9.2 – 9.5	6.2 – 12.0	8.1	9.0
$C_{18:1}$	20.4 - 21.2	22.6 – 26.3	11.4 – 17.3	9.3 – 25.2	35.1	12.9
$C_{20:1}$	0.8	7.6 – 7.7	3.2 – 8.1	7.3 – 19.9	5.8	12.1
$C_{22:1}$	-	4.5 – 5.2	3.6 – 7.8	6.9 – 30.6	4.9	13.9
$C_{18:2}$	4.1 - 5.5	1.1 – 1.4	1.3 – 2.5	0.1 – 2.9	0.7	1.28
$C_{18:3}$	4.0 - 6.0	1.3	0.9 – 1.3	0.3 – 1.1	0.3	1.1
$C_{18:4}$	2.8 – 3.2	1.9 – 2.5	2.0 – 2.9	1.1 – 2.8	0.7	2.5
$C_{20:4}$	5.3 – 7.1	0.5 – 1.0	1.6 – 2.5	0.3 – 1.2	0.3	0.9
$C_{20:5}$	5.6	11.3 - 13.6	9.6 – 16.9	3.9 – 15.2	5.3	7.6
$C_{22:5}$	4.1 - 8.7	1.7	2.5 – 2.8	0.3 – 1.3	0.2	0.6
$C_{22:6}$	9.3 – 15.1	5.1 – 10.6	8.5 – 12.9	2.0 – 7.8	1.5	7.7

6.1.2 Identification of marine oils in the archaeological record

Aristotle was the first to mention fishing in *Historia Animalium*, dating from the 4th century BC (Béarez, 1998), but it is likely that fishing has been practised since ancient times. For example, cod fishing has been established as being of importance in prehistoric Scotland from the Neolithic period (*ca.* 3500 to 2000 BC; Barrett *et al.*, 1999). Historical and archaeological evidence, including fish bones and fish hooks, suggests that long-range trade of preserved fish and of fish oil developed during the Viking Age, but became more important during medieval times (*ca.* AD 1050 to AD 1468; Barrett *et al.*, 1999). Such activities would have left organic residues in unglazed ceramic vessels.

Early work on the identification of degraded marine oils in the archaeological record focused on the analysis of fatty acids after saponification of the residue, and comparison with experimentally degraded marine oils (Patrick *et al.*, 1985; Malainey *et al.*, 1999) and fresh marine oils (Morgan *et al.*, 1983; Patrick *et al.*, 1985; Morgan *et al.*, 1992; Malainey *et al.*, 1999). Identification relied on comparison of the fatty acids relative abundance, but also on the identification of characteristic long-chain compounds, including $C_{20:1}$, $C_{22:1}$, $C_{24:1}$ (Morgan *et al.*, 1983; Patrick *et al.*, 1985; Morgan *et al.*, 1992; Malainey *et al.*, 1999). More recently, analysis of archaeological residues by high temperature-gas chromatography enabled the recovery of a highly complex mixture that was not totally resolved on such column (Dudd, 1999). Such complex mixtures are thought to arise from the oxidation of polyunsaturated fatty acids of the type characteristically found in fish oils, yielding polar compounds unresolvable by high temperature-gas chromatography (Dudd, 1999). $\delta^{13}C$ of the $C_{16:0}$ and $C_{18:0}$ fatty acids forming part of the mixture compared favourably with the values obtained for fresh fish oils (Dudd, Copley and Evershed, unpublished results).

6.1.3 Aims of the chapter

Archaeological and historical evidences suggest that fish products must have played an important role in Antiquity. The high degree of unsaturation of fish oils make them very susceptible to decay, especially oxidation, during vessel burial, with the result that their presence is rarely reported in the archaeological record. The aims of this chapter are therefore:

(i) To submit fish oil (cod liver oil was chosen because of the importance of cod in Antiquity) absorbed in unglazed ceramics to experimental decay under a range of conditions chosen to promote a range of chemical and microbiological degradation reactions.

(ii) To investigate the fate of the long-chain polyunsaturated compounds characteristic of fish oils during vessel burial.

6.2 Experimental decay of fish oils under laboratory conditions (Experiments 15, 16 and 17)

6.2.1 Potsherd organic carbon content

Cod liver oil consists mainly of triacylglycerols containing long-chain polyunsaturated fatty acyl moieties which makes it un-amenable to high temperature-gas chromatography. As a consequence, it was not possible to quantify the total lipid extract from potsherds containing degraded cod liver oil by HTGC as was done during the experimental decay of olive oil and dairy fats, and for archaeological samples. Thus, elemental analysis was used to obtain information regarding the organic carbon content of the potsherds during the experimental decay of cod liver oil. The results are summarized in Figure 6.1. During experiments 15 and 16 (Figures 6.1a and 6.1b), there was a marked decrease in the organic carbon content with time, from 0.9 to 0.2% after 148 and 286 days, respectively. During experimental 17, the organic carbon content remained quite constant at around 0.8%. During these 3 experiments, a significant concentration of organic carbon remained in the potsherd after solvent-extraction. It accounted for up to 0.2% organic carbon, and remained relatively constant with time, and was most likely represented by unextractable organic components strongly absorbed in the ceramic matrix.

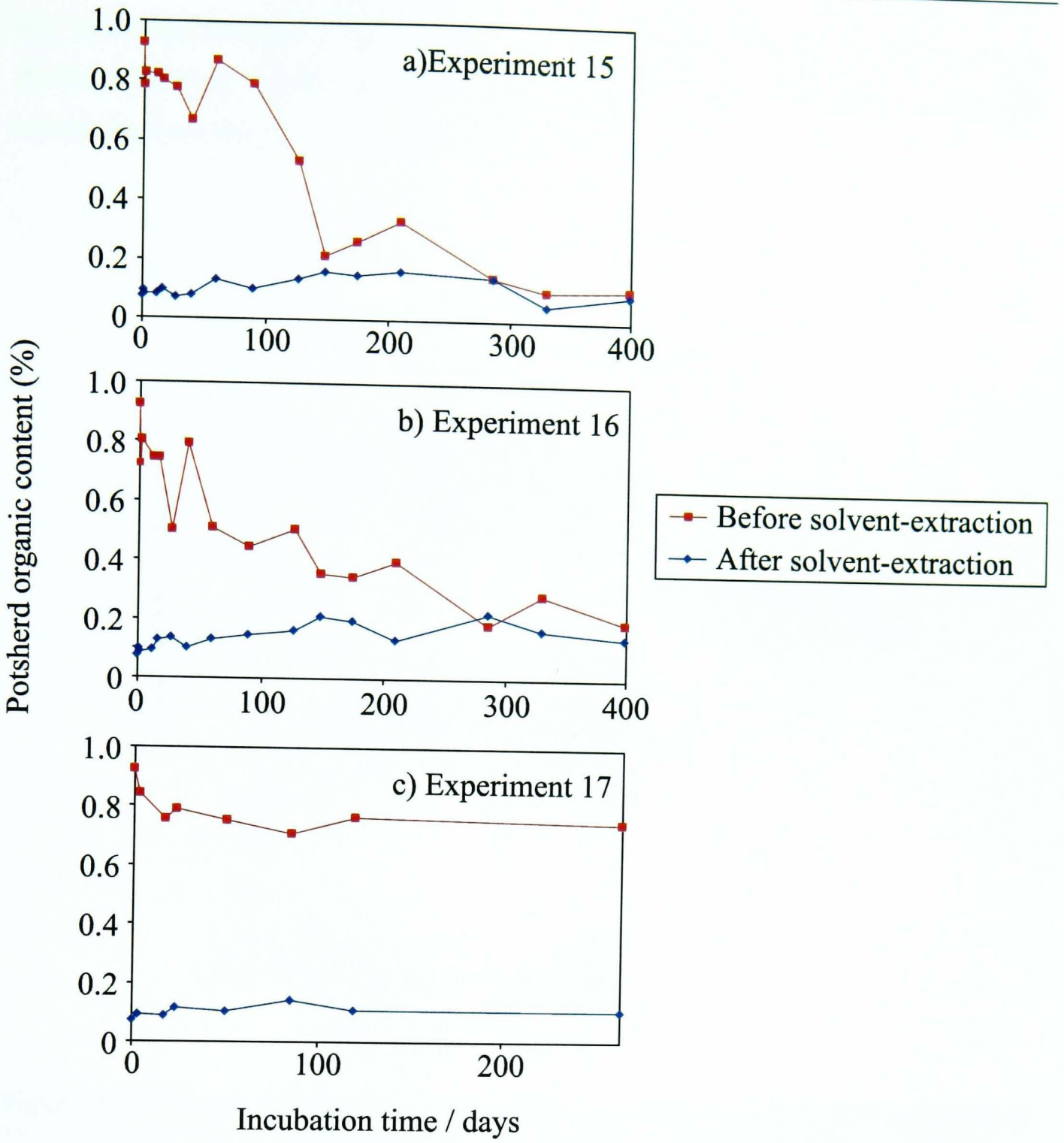

Figure 6.1: Potsherd organic carbon content before and after solvent-extraction during the experiments a) 15, b) 16 and c) 17 investigating the experimental decay of cod liver oil under laboratory conditions.

6.2.2 Acid and neutral fractions

The total lipid extracts extracted from the potsherds sampled during the experiments investigating the experimental decay of cod liver oil were submitted to acid-neutral separation (Section 2.5.1). The acid fraction consisted mainly of free fatty acids, the neutral fractions of triacylglycerols, with di- and monoacylglycerols present in much lower abundance. After addition of an internal standard ($C_{21:0}$), the acid fraction was methylated (Section 2.5.3) and trimethylsilylated (Section 2.5.5) and analysed by GC (Section 2.6.1) and GC-MS (Section 2.6.2) on a CPSil-5 column. The neutral fraction

was saponified (Section 2.5.2) to yield fatty acids that were treated in the same way as the free fatty acids. Figure 6.2 shows the concentrations of lipid recovered in the acid and neutral fractions during experiments 15, 16 and 17.

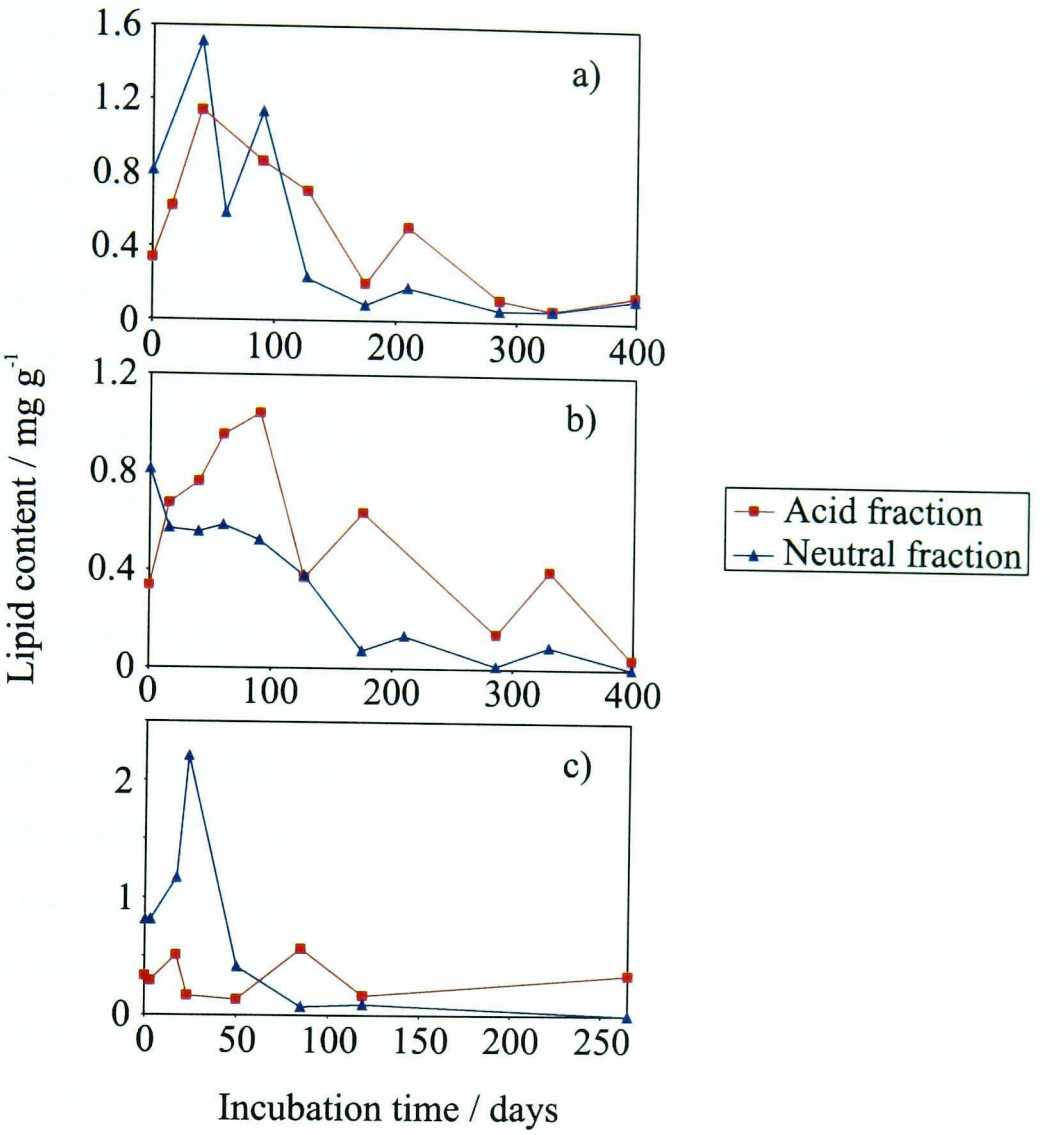

Figure 6.2: Changes in the acid and neutral fraction content during the experiments a) 15, b) 16 and c) 17 investigating the decay of cod liver oil under laboratory conditions.

During experiments 15 (moist mushroom compost) and 16 (dry mushroom compost), the lipid content in the neutral fraction decreased regularly with time [if allowance is made for the initial difference in lipid content due to the difference in potsherd size (Section 3.2.1.3)], whereas the lipid content of the acid fraction first increased and then decreased. The trends seen in these two experiments were very similar, and are consistent with the mechanism of triacylglycerol hydrolysis followed by free fatty acids degradation. During experiment 17 (no mushroom compost), the lipid content of the neutral fraction decreased very rapidly, but the lipid content of the acid fraction remained low. During this experiment, degradation of the triacylglycerols in the neutral fraction did not yield free fatty acids exclusively.

6.2.3 Fatty acid composition of the acid and neutral fractions

6.2.3.1 Experiment 15

During the experiment 15, the free fatty acid distributions remained quite constant and consistent with the composition of the cod liver oil used as a substrate (Table 6.2) until 127 days of incubation. The free fatty acid distribution was then dominated by $C_{18:1}$, $C_{18:0}$ and $C_{20:1}$. After 148 days of incubation, there weas a sharp decrease in the relative abundance of the unsaturated fatty acids $C_{16:1}$, $C_{18:1}$, a moderate decrease in the relative abundance of $C_{20:1}$, and a sharp increase in the relative abundance of $C_{22:1}$. This pattern continued throughout the experiment, such that, after 399 days of incubation, the free fatty acid distribution was dominated by $C_{22:1}$. Such a dominance of the unsaturated $C_{22:1}$ is unexpected, and may be due to either a later release from the triacylglycerols compared to the other compounds, or to the higher degree of preservation of this compound.

The fatty acid composition of the neutral fraction remained similar to that of the cod liver oil used in the experiment until after 330 days of incubation, when there were slight decreases in the relative abundance of the unsaturated compounds $C_{16:1}$, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$, and an increase in the relative abundance of $C_{16:0}$. Furthermore, the saturated fatty acids, $C_{20:0}$ and $C_{23:0}$, were also detected in this fraction. After 399 days of incubation, $C_{16:1}$ and $C_{22:1}$ were not detected in the fraction, and there was a significant increase in the relative abundance of $C_{14:0}$.

6.2.3.2 Experiment 16

During experiment 16, the free fatty acid distribution (Table 6.3) remained constant and similar to the fatty acid composition of the cod liver oil used until after 399 days of incubation, when no unsaturated compounds were detected. The fatty acid composition of the neutral fraction remained similar to that of the cod liver oil used until after 330 days of incubation, when there was an unexpected increase in the relative abundance of the unsaturated $C_{18:1}$ (to 55%). After 399 days of incubation, all the unsaturated fatty acids became very depleted, and the neutral fraction was dominated by $C_{16:0}$.

Table 6.2: Fatty acid distributions in the acid and neutral fractions during the experimental decay of cod liver oil (Experiment 15).

	Relative abundance (%)								
	Acid fraction						Neutral fraction		Pure fish oil
Incubation time / day	127	148	175	286	330	399	330	399	
Fatty acids									
$C_{14:0}$	4.4	6.3	2.2	3.5	0.0	2.0	3.6	23.9	7.5
$C_{15:0}$	0.8	0.6	1.1	2.2	2.7	1.6	0.0	0.0	0.0
$C_{16:1}$	8.8	1.5	1.2	0.0	0.0	0.0	2.9	0.0	8.3
$C_{16:0}$	17.5	25.2	23.4	22.8	11.8	16.0	31.9	27.3	25.2
$C_{17:0}$	1.4	1.0	2.7	3.8	4.4	4.2	0.0	0.0	0.7
$C_{18:1}$	40.2	12.3	15.6	12.8	6.9	16.7	18.2	25.0	32.5
$C_{18:0}$	4.3	8.1	10.4	8.9	7.1	10.1	8.3	7.6	6.3
$C_{20:1}$	14.0	12.5	14.5	7.5	5.1	9.9	6.9	16.1	10.9
$C_{20:0}$	0.0	0.0	0.0	2.5	4.7	0.0	2.1	0.0	0.0
$C_{22:1}$	8.7	30.7	28.8	31.7	43.8	39.6	6.2	0.0	7.4
$C_{23:0}$	0.0	0.0	0.0	0.0	0.0	0.0	19.9	0.0	0.0

Table 6.3: Fatty acid distribution in the acid and neutral fractions during the experimental decay of cod liver oil (Experiment 16).

	Relative abundance (%)					
	Acid fraction		Neutral fraction			
Incubation time / day	330	399	286	330	399	Pure fish oil
Fatty acids						
$C_{14:0}$	6.9	4.5	6.7	0.0	13.1	7.5
$C_{15:0}$	1.3	1.3	1.1	1.5	2.4	0.0
$C_{16:1}$	6.3	0.0	4.1	6.7	0.0	8.3
$C_{16:0}$	30.9	45.9	25.0	26.8	55.5	25.2
$C_{17:0}$	1.9	3.0	1.3	1.5	3.7	0.7
$C_{18:1}$	14.6	7.9	24.9	55.0	3.8	32.5
$C_{18:0}$	9.0	37.4	6.5	8.5	15.8	6.3
$C_{20:1}$	15.4	0.0	7.7	0.0	0.0	10.9
$C_{22:1}$	13.7	0.0	11.1	0.0	5.5	7.4

6.2.3.3 Experiment 17

During experiment 17 (Table 6.4), the free fatty acid distribution remained consistent with the fatty acid composition of the cod liver oil used as substrate for only 17 days of incubation after which there was a significant decrease in the relative abundance of the unsaturated fatty acids $C_{16:1}$, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$. After 265 days of incubation, $C_{18:1}$ was the only unsaturated compound detected, and was very depleted in its abundance compared to the cod liver oil used as substrate (only 1.8% compared to 32.5% at the

beginning of the experiment). A similar trend was observed in the fatty acid composition of the neutral fraction.

Table 6.4: Fatty acid distributions in the acid and neutral fractions during the experimental decay of cod liver oil (Experiment 17).

	Relative abundance (%)						
	Acid fraction			Neutral fraction			
Incubation time / day	3	17	265	3	17	265	Pure fish oil
Fatty acid							
$C_{14:0}$	7.3	16.0	16.8	4.0	18.9	14.7	7.5
$C_{15:0}$	1.3	3.8	3.6	0.8	3.3	0.0	0.0
$C_{16:1}$	5.6	0.0	0.0	5.3	0.0	0.0	8.3
$C_{16:0}$	36.0	60.6	60.0	20.2	60.5	68.0	25.2
$C_{17:0}$	2.3	2.8	3.2	1.4	0.0	0.0	0.7
$C_{18:1}$	26.7	3.1	1.8	32.8	4.3	5.8	32.5
$C_{18:0}$	10.6	13.6	14.6	6.5	13.0	11.6	6.3
$C_{20:1}$	7.5	0.0	0.0	15.8	0.0	0.0	10.9
$C_{22:1}$	2.9	0.0	0.0	13.4	0.0	0.0	7.4

6.2.4 Oxidation products

Oxidation products, such as α,ω-dicarboxylic acids and hydroxy acids would be expected to arise from oxidation of the unsaturated components of cod liver oil, resulting in the depletion of the unsaturated fatty acids. Table 6.5 shows the relative abundance of the unsaturated compounds in the acid and neutral fractions during experiments 15, 16 and 17. During experiment 15, the relative abundance of unsaturated compounds remained high throughout appearing not to be affected by oxidative processes such that no oxidation products were detected in the acid or neutral fractions. During experiments 16 and 17, unsaturated compounds were very depleted, and oxidation products were expected in the acid and/or neutral fractions. Oxidation products were only detected in the acid fraction from experiment 17 after 85 days of incubation (Figure 6.3). The absence of oxidation products from the experiments 15 and 16, and from other samples from the experiment 17 might be explained by their incorporation into "bound" fractions,

Table 6.5: Total relative abundance of the unsaturated compounds found in the acid and neutral fractions during experiments 15 and 16 (after 399 days of incubation) and 17 (after 265 days of incubation) investigating the decay of fish oil under laboratory conditions.

	Relative abundance (%)			
Experiment	15	16	17	Pure fish oil
Incubation time / day	399	399	265	
Acid fraction	66.1	7.9	1.8	-
Neutral fraction	41.2	9.4	5.8	59.0

Figure 6.3 shows the GC trace of the acid fraction recovered after 85 days of incubation during experiment 17. The acid fraction is dominated by fatty acids in the proportions described in the Section 6.2.3.3. In the early part of the chromatogram are found a range of α,ω-dicarboxylic acids, ranging from C_8 to C_{10}, dominated by the C_9 components. The branched fatty acid 4,8,12-trimetyltridecanoic acid, a compound commonly found in marine oils is also present (see Section 6.2.5). In the later part of the chromatogram, the residue contains a range of dihydroxy acids, namely 9,10-dihydroxyhexadecanoic acid (*m/z* 187 and 259), 9,10 and 11,12-dyhydroxyoctadecanoic acids (*m/z* 215 and 259 and *m/z* 187 and 287 respectively), 11,12-dihydroxyeicosanoic acid (*m/z* 215 and 287) and 11,12-dihydroxydocosanoic acid (*m/z* 243 and 287). These compounds are thought to be produced from unsaturated fatty acids, with the position of the hydroxy groups reflecting the original position of the double-bond. The compounds indicated in Figure 6.3 are therefore thought to be produced from $C_{16:1}\Delta^9$, $C_{18:1}\Delta^9$, $C_{18:1}\Delta^{11}$, $C_{20:1}\Delta^{11}$ and $C_{22:1}\Delta^{11}$. This agrees with the composition of the cod liver oil used in this experiment which contained one $C_{16:1}$ isomer ($C_{16:1}\Delta^9$), two $C_{18:1}$ isomers [$C_{18:1}\Delta^9$ (70 %), $C_{18:1}\Delta^{11}$ (20%)], and one $C_{20:1}$ isomer ($C_{20:1}\Delta^{11}$; the double-bond position in $C_{22:1}$ was not determined).

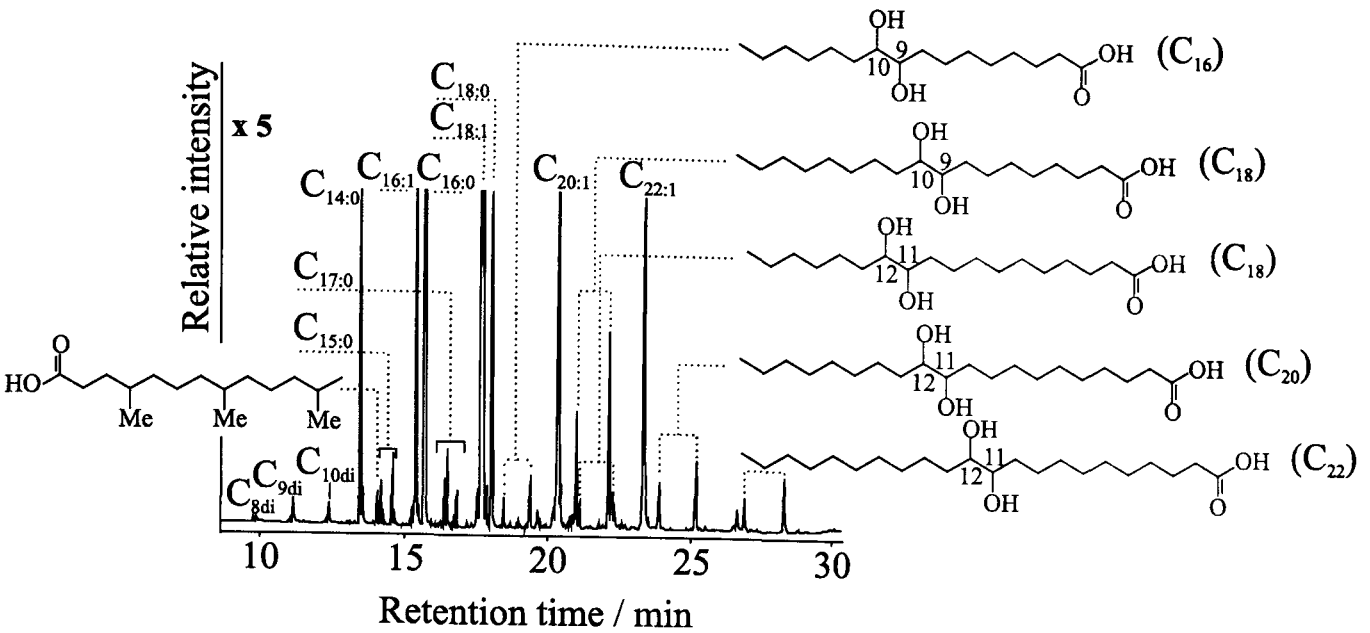

Figure 6.3: Partial GC trace of the acid fraction recovered after 85 days of incubation during the experiment 17 investigating the decay of fish oil under laboratory conditions. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds, C_{xdi} = α,ω-dicarboxylic acid with x carbon atoms, (C_y) = indicated the number of acyl carbon (y) in the structure on the left hand side. All compounds present as Me esters, TMS ethers. The vertical scale was expanded 5x to reveal the minor components of the residue. Analysis was performed on a 50 m x 0.32 mm i.d. CPSil-5 CB column (Section 2.6.1).

6.2.5 Isoprenoid compounds

Two isoprenoid compounds, namely 4,8,12-trimethyltridecanoic acid and 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) were identified in the acid and neutral fractions of the degraded fish oil, and accounted for 1.2% of the fresh cod liver oil used as substrate in the experiments. The retention time of these compounds when prepared as their TMS esters and analysed on a polar column are shown in Figure 6.4. These compounds were identified by their characteristic mass spectra, shown in Figure 6.5. These compounds were detected in the experiments 15 and 16 until 286 days of incubation, and accounted for between 1.9 and 24.4 % of the residues, but were only identified in the acid fraction recovered after 85 days of incubation during the experiment 17. These compounds were not detected in the first residues recovered during the first stages of Experiment 17 because of their low abundance compared to the other components of the residues. These compounds are usually present in the lipids of land and aquatic animal (Gunstone *et al.*, 1986) and have been reported in several fishes (e.g. Kang *et al.*, 1998).

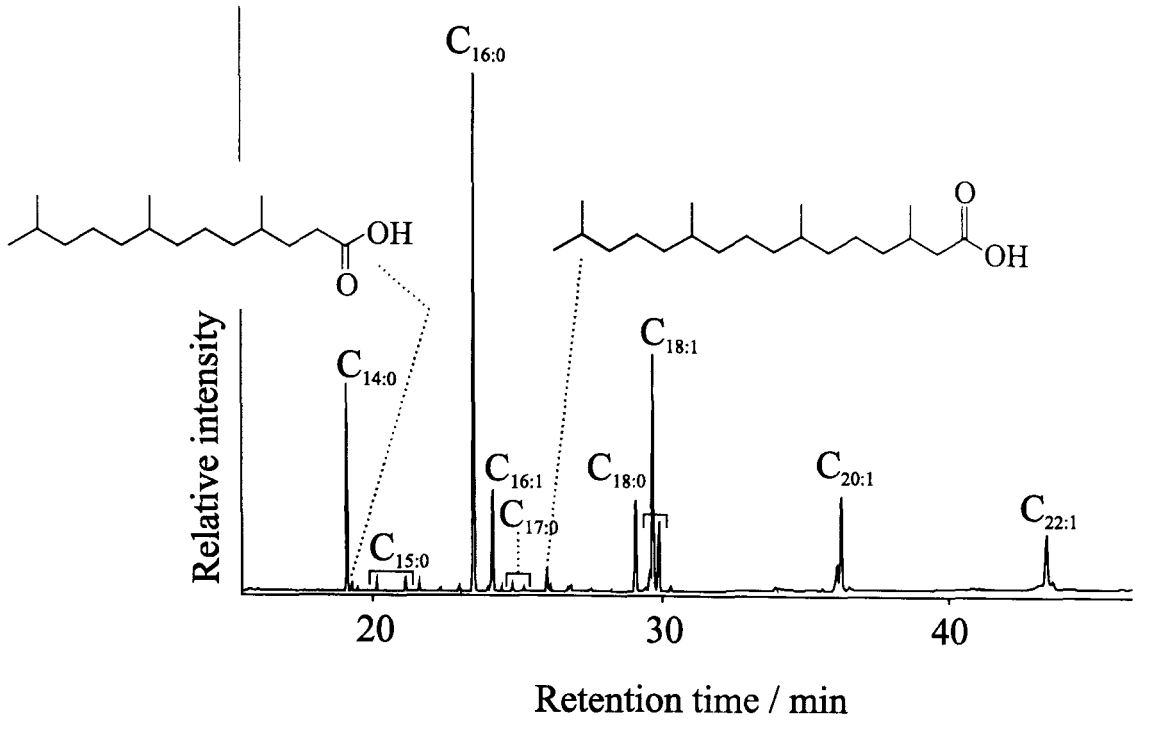

Figure 6.4: Partial GC trace of the neutral fraction of fresh cod liver oil. Peak identities: $C_{m:n}$ = fatty acid with m carbon atoms and n double-bonds. All compounds were present as their TMS esters. Analysis was performed on a 25 m x 0.32 mm i.d. CP-Wax column (section 2.7.1).

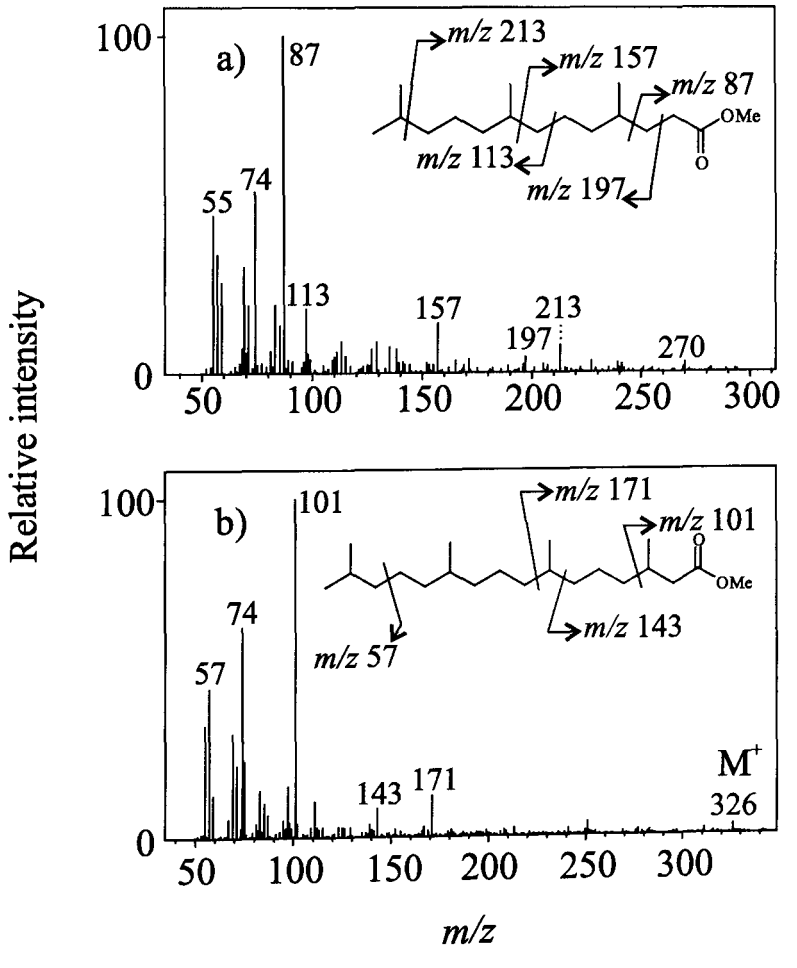

Figure 6.5: Mass spectral fragmentation of a) 4,8,12-trimethyltridecanoic acid, and b) 3,7,11,15-tetramethylhexadecanoic acid. Both compounds were determined as their Me esters.

6.2.6 "Bound" fractions

Only low abundance of lipids were recovered by alkaline hydrolysis of the extracted potsherds during the experiments 15, 16 and 17, except for 2 samples: experiment 15, after 399 days of incubation, and experiment 17, after 85 days of incubation. The compositions of the "bound" fractions recovered from these samples are shown in Table 6.6. Both residues were dominated by fatty acids, mainly $C_{16:0}$ and $C_{18:0}$. The unsaturated $C_{18:1}$ was very depleted compared to the original composition of the cod liver oil used in these experiments. No fatty acids containing more than 18 carbon atoms were detected in these residues. The "bound" fraction also contained a range of α,ω-dicarboxylic acid, ranging from C_8 to C_{10}, and dominated by C_{10} (experiment 15), and C_9 (experiment 17).

Table 6.6: Composition of the "bound" fractions extracted after 399 days of incubation during experiment 15, and 265 days of incubation during experiment 17, compared to the fatty acid distribution in the fresh cod liver oil used as substrate in these experiments.

	Relative abundance (%)		
Experiment	15	17	Pure fish oil
Incubation time / day	399	85	
α,ω-dicarboxylic acids			
C_8	0.0	2.1	0.0
C_9	2.3	7.3	0.0
C_{10}	11.4	4.5	0.0
Fatty acids			
$C_{14:0}$	5.8	10.0	7.5
$C_{15:0}$	0.0	0.9	0.0
$C_{16:0}$	55.3	54.8	25.2
$C_{17:0}$	3.0	3.3	0.7
$C_{18:1}$	6.9	2.8	32.5
$C_{18:0}$	15.3	14.3	6.3

6.3 Conclusions

The effects of decay on cod liver oil during incubation under "hydrolytic" and "oxidative" conditions were comparable to those on olive oil (Section 4.6) and on dairy fats (Section 5.4) and will be detailed in Chapters 7 and 8, respectively. The main conclusions are:

(i) In all decay experiments, an overall decrease was seen in the organic carbon content of the potsherd, paralleled by a decrease in the lipid content of the neutral fraction of the total lipid extract.

(ii) The fatty acid composition of the acid and neutral fractions of the degraded cod liver oil residues showed a reduced abundance of the longer chain and more unsaturated fatty acids. The extent of the decay of these more labile components was governed by the incubation conditions.

(iii) Only limited quantities of oxidation products were recovered from either the "bound" or solvent-extractable fractions. However, a series of dihydroxy acids whose structures reflected those of their precursor unsaturated fatty acids was identified in one degraded cod liver oil residue.

(iv) Two characteristic isoprenoid acids were identified in the cod liver oil residues, and their presence might be used in the identification of degraded marine oil residues in archaeological pottery.

CHAPTER 7: DISCUSSION: DEGRADATION OF LIPID RESIDUES UNDER "HYDROLYTIC" CONDITIONS

7.1 Identification of the processes affecting lipid residues under "hydrolytic" conditions

7.1.1 Ester hydrolysis

Chemical and enzymatic ester hydrolysis greatly affects lipids during oil and fat storage or processing (Davídek *et al.*, 1990), in soils (Hita *et al.*, 1996) and in archaeological artefacts (Charters, 1996; Dudd, 1999) and was expected to be important during the experimental decay of lipid residues (Charters, 1996; Dudd, 1999). Triacylglycerol hydrolysis proceeds stepwise by sequential removal of acyl moieties from the glycerol backbone to yield mono- and diacylglycerols and free fatty acids (Section 1.3.1; Morrison, 1992). Hydrolysis proceeds faster once one acyl moiety has been removed from the glycerol backbone such that partial acylglycerols do not accumulate (Davídek *et al.*, 1990). In soils (Hita *et al.*, 1996) and in experimental ceramics (Charters, 1996; Dudd, 1999), triacylglycerol hydrolysis is usually accompanied by an overall decrease in lipid content, which is thought to be due to lipid consumption by micro-organisms by β-oxidation (Evershed, 1992). Monitoring of the composition of the absorbed residues and of the potsherd lipid content revealed similar trends in the tri-, di-, monoacylglycerol and free fatty acid relative abundances during the experimental decay of olive oil (Section 4.2.1), cow butter (Section 5.3.1) and goat butter (Section 5.3.2) under conditions that were qualified "hydrolytic" and all had in common low (5° C) to intermediate (25° C) incubation temperature, and the presence of water in the burial environment. Identification of ester hydrolysis as a process affecting cod liver oil absorbed in unglazed ceramics under identical conditions was more difficult to assess as the triacylglycerol relative abundance in the degraded residues was not monitored, but it is extremely likely that ester hydrolysis was the main process affecting absorbed fish oil under "hydrolytic" conditions (Section 6.2.2). Finally, similar trends were observed during the experimental decay of dairy fats under field conditions (Section 5.2) aimed at replicating the conditions encountered by archaeological ceramics.

7.1.2 Unsaturated lipid oxidation

Unsaturated lipid oxidation is thought to be responsible for the preferential depletion of unsaturated lipids in the archaeological record but oxidation products have been identified most frequently associated with artefacts excavated from arid sites (Gülaçar *et al.*, 1989, 1990; Regert *et al.*, 1998 and in press; Bland, 1999). "Hydrolytic" conditions, characterised by low temperature and a moist environment, were not expected to promote oxidation, but oxidation products were identified during the experimental decay of olive oil. Indeed, a series comprising four unsaturated hydroxyacids was identified in most of the olive oil residues, together with 9,10-dihydroxyoctadecanoic acid (Section 4.3.1.3). Radical oxidation of oleic acid, the major fatty acid in olive oil, is well documented (Frankel, 1998 and references therein) and yields, in a first step, the four unsaturated hydroperoxides shown in Figure 7.1 (Frankel, 1998 and references therein). The spontaneous reduction of hydroperoxides to the more stable hydroxy derivatives has previously been reported during sample fractionation (Saeed and Howell, 1999), and it is likely that the unsaturated hydroxyacids observed in the olive oil residues resulted from spontaneous reduction either during burial, with the clay acting as a catalyst (Shimoyama *et al.*, 1993), or during sample preparation. Furthermore, the presence of these four isomers demonstrates that oxidation proceeded *via* a radical mechanism as photosensitised or enzymatic oxidation would have yielded different isomers (see Sections 1.3.4.2 and 1.3.4.3). The presence of 9,10-dihydroxyoctadecanoic acid has been previously reported in degraded adipose fat in adipocere (Evershed, 1990 and 1992) and mummies (Buckley and Evershed, 1999, Gülaçar *et al.*, 1989 and 1990) and in archaeological (Regert *et al.*, 1998a and 1998b; Bland, 1999) and experimental (Bland, 1999) ceramics. 9,10-dihydroxyoctadecanoic is a known product of $C_{18:1}\Delta^9$ and is thought to arise either from (i) dihydroxylation of the double-bond (Gülaçar *et al.*, 1990; Bland, 1999), (ii) secondary reaction between $C_{18:1}$ and the hydroperoxides (Frankel *et al.*, 1997), or (iii) from the hydroperoxides themselves (Frankel *et al.*, 1997).

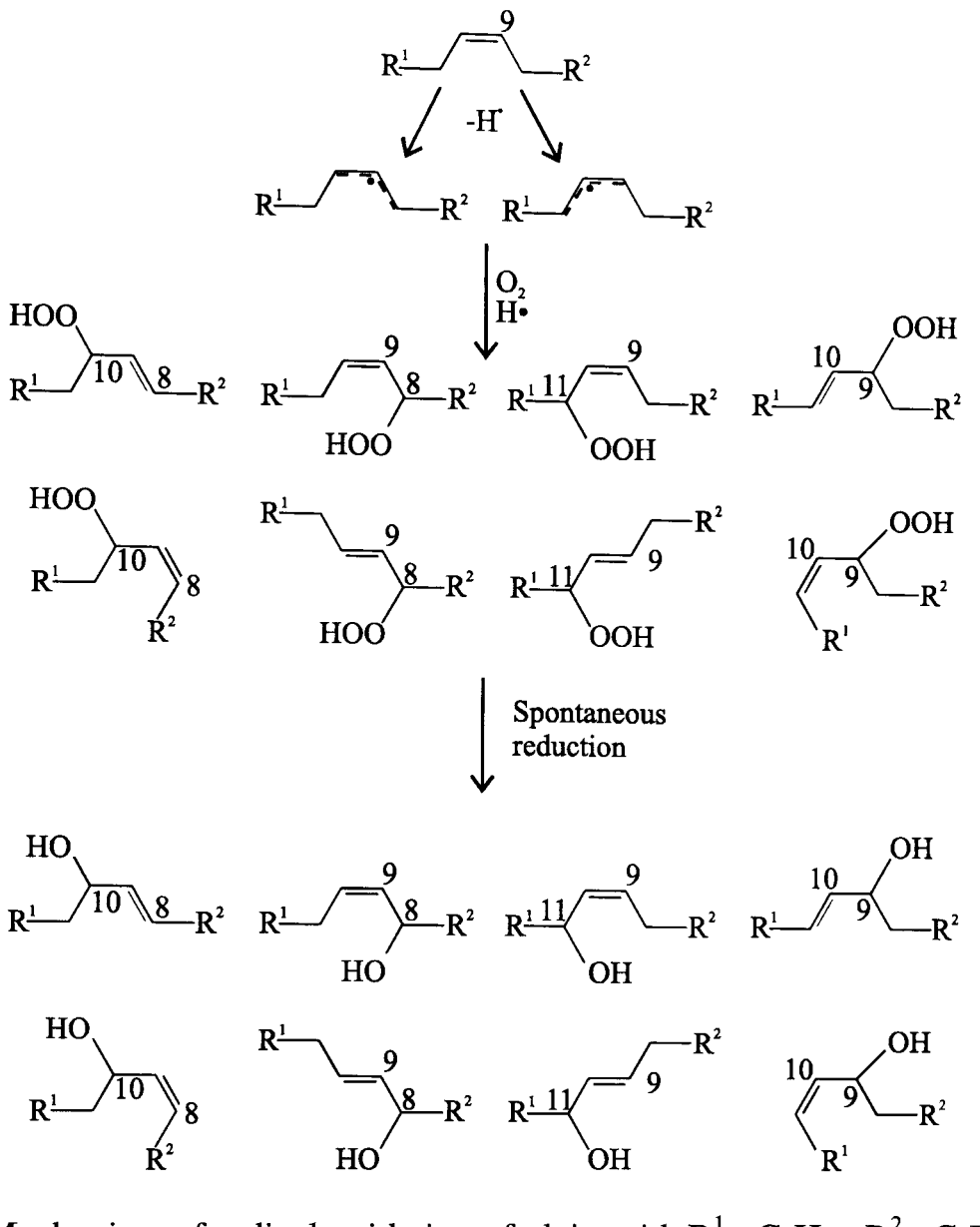

Figure 7.1: Mechanism of radical oxidation of oleic acid. $R^1 = C_7H_{15}$, $R^2 = C_7H_{13}O_2$.

No oxidation products were detected during the experimental decay of dairy fats under "hydrolytic" conditions in the laboratory or under field conditions. Dairy fats are not a good substrate for radical oxidation because both their low degree of unsaturation and the antioxidant activity of some milk components (whey, casein and some enzymes) prevent the propagation step of radical oxidation (Angulo *et al.*, 1997; Shiota *et al.*, 1999).

Finally, fish oils are very unsaturated and were expected to be a good substrate to study oxidative processes. The absence of oxidation products from degraded cod liver oil residue can be explained either by (i) the strong association of these compounds in the "bound" fraction, or by (ii) oxidation taking place to such a large extent, resulting in the production of volatile oxidation products which would have been lost from the incubation experiments.

7.2 Rate and extent of degradation

7.2.1 Ester hydrolysis

The rate and extent of ester hydrolysis was strongly influenced by the incubation conditions. A low incubation temperature or the absence of water inhibited ester hydrolysis. Experiments conducted at moderate temperature were the fastest, whereas higher temperature promoted lipid oxidation. Likewise, addition of a moderate amount of water to the incubation environment increased the rate of hydrolysis, whereas saturation did not promote further increases. These observations are consistent with biological mediated hydrolysis. Indeed, in soil, triacylglycerol hydrolysis is known to proceed mainly by biological processes, especially in neutral soils [Hita *et al.*, 1996; both the mushroom compost (pH = 7.2) and the burial soil (pH= 6.7) had a pH close to neutrality]. Furthermore, fungal growth on the surface of the mushroom compost was observed during most of the experiments, as shown in Figure 7.2. Finally, degradation of intact milk fat, and of heat-treated milk fat [in which the natural lipases would have been denatured (Stead, 1986; Wandsnider, 1997)] proceeded at the same rate, so it is very likely that microorganisms present in the incubations were responsible for decay.

Figure 7.2: Flask containing a potsherd dosed in olive oil and mushroom compost incubated for one year during the experiment 4.

Soil moisture governs the rate and extent of soil organic matter decay (Eglinton and Logan, 1991; Paul and Clark, 1996; Schimel *et al.*, 1999, Wachendorf *et al.*, 1997; Leirós *et al.*, 1999) and was expected to influence the rate and extent of the degradation of absorbed lipid residues. The amount of water in the burial environment affects degradation in 2 ways: (i) it controls the structure and activity of the microbial community (Schimel *et al.*, 1999) and is necessary for bacterial activity, and (ii) it controls many soil properties, such as pH, redox potential, aeration, etc (Paul and Clark, 1996). Furthermore, the bacterial decay of organic matter is inhibited in soils with major water deficits (Parshotam *et al.*, 2000; Bottner *et al.*, 2000; Wandsnider, 1997). During the experimental decay of lipid residues described herein, dry conditions (dry mushroom compost) consistently inhibited decay (see for example Experiment 1). Addition of water to the mushroom compost promoted decay by enhancing bacterial activity (Alexander, 1999). Waterlogging of the mushroom compost did not increase the rate of decay, probably because high water contents limited the avalaibility of oxygen (Bohn *et al.*, 1979; Killops and Killops, 1993; Alexander, 1999).

The rate and extent of hydrolysis was also dependant on the incubation temperature. A moderate increase in temperature (from 7°C to 25°C) promoted hydrolysis but warmer conditions were more favourable to oxidation. This is consistent with the fact that chemical reactions are promoted by higher temperatures whereas biological reactions show an optimum range of temperature (Gray, 1989; Magid *et al.*, 1999; Leirós *et al.*, 1999), usually between 30 and 40°C for mesophile bacteria (Atlas, 1986). Low temperatures during experimental decay under laboratory or field conditions inhibited decay, whereas the moderate temperature used in some experiments fell into the optimum range for many saprophytic organisms and promoted decay. Higher temperatures were above the optimum range, and inhibited bacterial activity (but promoted chemical oxidation). Overall, these results clearly indicate that it is important to consider both the temperature and water content of the burial environment when assessing the preservation potential of a site (Cortez, 1998).

Anaerobic decay is usually thought to be slower than aerobic decay (DeLaune *et al.*, 1981) but the most important factor governing the rate of decay seems to be the structure of the substrate (Sun *et al.*, 1997). The aeration status however governs the structure and activity of microbial community involved in organic matter decay (DeLaune *et al.*, 1981). The anaerobic decay of lamb fat absorbed in unglazed ceramics was slower than

the aerobic decay (Charters, 1996; Dudd, 1999) but, in the experiments described herein, there was no significant difference between the aerobic (Experiment 5) and anaerobic (Experiment 7; Section 4.2.1) decay of olive oil. This observation might be explained by the very large variety and abundance of microorganisms in mushroom compost (Killops and Killops, 1993).

The rate and extent of ester hydrolysis was also influenced by the amount of lipid absorbed in the unglazed ceramics. This was particularly noticeable during the experimental decay of dairy fats under field (Section 5.2), and laboratory (Section 5.3) conditions. The milk fat was degraded much faster than the butter fat, and the goat butter faster than the cow butter, and the only difference in each set of experiments was the concentration of fat absorbed in the potsherds, given that the chemical composition of the substrates was very similar. In soils, high concentrations of fatty acids are known to slow down decomposition by forming waterproof films (Amblès *et al.*, 1994).

7.2.2 Unsaturated lipid oxidation

Unsaturated lipid oxidation only plays a minor role during the laboratory decay of absorbed lipid residues under "hydrolytic" conditions, and was only noticeable during the experimental decay of olive oil. The oxidation products described in Section 8.1.2 were identified in most degraded olive oil residues. The presence of 4 different isomers indicates that these compounds were produced by chemical oxidation of $C_{18:1}$, as biological oxidation would have produced only one isomer (German *et al.*, 1992). Unsaturated lipid oxidation was most important during experiments conducted at high temperatures, and containing a low to moderate amount of water in the incubations. The importance of lipid oxidation in the degradation of lipid residues absorbed in unglazed ceramics has been recently demonstrated through the analysis of ceramics (Regert *et al.*, 1998; Bland, 1999) and mummies (Gülaçar *et al.*, 1989 and 1990; Buckley and Evershed., 1999) mostly excavated from arid sites, which confirms the importance of low levels of water and high temperature in promoting oxidative decay. Analysis of the "bound" fraction associated with ceramics dosed with olive oil and incubated under "hydrolytic" conditions revealed the presence of hydroxy and ketoacids in low concentrations (Section 4.3.2). These compounds may have been produced by radical oxidation of the unsaturated fatty acids but it is more likely that they were produced by microbial reworking of the unsaturated fatty acids (Gunstone et al., 1986), which has

been reported to take place in soils (Schroepfer, 1966; Barakat *et al.*, 1994) and during adipocere formation (Kawamura and Gagosian, 1988; Takatori and Yamaoka, 1977a, 1977b; Kerwin and Torvik, 1996).

7.3 Preservation of the chemical and isotopic composition of the absorbed residues

7.3.1 Triacylglycerols

During all experiments, the triacylglycerol fraction of the residues became depleted. The triacylglycerol distribution in the degraded olive oil residues was not altered during experimental decay under "hydrolytic" conditions, as all triacylglycerols were hydrolysed at similar rates, independently of their molecular weight or degree of unsaturation. During the experimental decay of dairy fats, however, the triacylglycerol distributions were altered as the shorter-chain compounds were preferentially depleted compared to their longer-chain counterparts. This has been reported previously (Dudd *et al.*, 1998; Dudd, 1999; Dudd and Evershed, 1998) during the experimental decay of milk fat under laboratory conditions. Short-chain acyl moieties are more susceptible to hydrolysis than their longer-chain counterparts because: (i) they are more polar than their longer-chain counterparts (Davídek *et al.*, 1989), (ii) they are usually found at the *sn*-3 position of triacylglycerols, where limited steric hindrance allows rapid hydrolysis, and, (iii) some lipases exhibit preference for short-chain acyl moieties (Stead, 1986; Choi and Jeon, 1993). As a result, triacylglycerol distribution in degraded dairy fats lack the low-molecular weight components, and resemble degraded animal fats (Dudd *et al.*, 1998; Dudd and Evershed, 1998; Dudd, 1999).

The fate of the triacylglycerol was not directly monitored during the experimental decay of cod liver oil, but it was possible to estimate their fate by looking at the fatty acid distribution of the neutral fraction, which comprises mainly triacylglycerols. During the experiment conducted under "hydrolytic" conditions (Section 6.2.3.1), the neutral fraction became slightly more saturated, probably as the unsaturated acyl moieties were preferentially degraded *via* oxidative processes.

7.3.2 Degradation products

7.3.2.1 Free fatty acids

During the experiments conducted under "hydrolytic" conditions,, the free fatty acid distribution generally reflected that of the original substrate fatty acids. However, the relative abundance of: (i) the short-chain fatty acids, and (ii) the unsaturated fatty acids, was significantly different between the free fatty acid fraction of the degraded residues and the total fatty acid fraction of the substrate.

During the experimental decay of dairy fats, the short-chain compounds were significantly depleted compared to their relative abundance in the original substrate. This has been previously reported and is thought to be due to the higher water solubility of the shorter-chain fatty acids compared to the longer-chain homologues (Dudd *et al.*, 1998; Dudd and Evershed, 1999; Dudd, 1999; Malainey *et al.*, 1999).

During all experiments conducted under "hydrolytic" conditions, the unsaturated fatty acids were preferentially degraded compared to their saturated homologues, probably because of minor oxidative processes. This preferential degradation has been reported in soils (Stefanova and Disnar, 2000) and ceramics (Charters, 1996; Malainey *et al.*, 1999a, b and c; Dudd, 1999) and was particularly noticeable during the decay of highly unsaturated substrates, such as olive oil or cod liver oil. However, very few oxidation products were detected, either in the solvent-extractable or "bound" fractions, except in degraded olive oil residues. Further analysis of the degraded dairy fats revealed that the distribution of the different unsaturated isomers was altered during decay as the *cis* configured fatty acids degraded more rapidly than the more stable *trans* configured fatty acids, and the resulting distributions were very similar to those observed in the archaeological ceramics from Eton (Section 3.3.1.2) and Crick (Section 3.32.2), and to distributions observed in degraded residues identified as degraded dairy fats (Dudd, 1999). During the experimental decay of dairy fats, the fatty acid fraction initially contained higher abundance of $C_{18:1}$ than would have been expected from the initial composition of the substrates. A similar trend have been reported during the experimental decay of lamb fat absorbed in unglazed ceramics (Dudd, 1999) and can by explain by the fact that: (i) this fatty acid is widespread in bacteria and could therefore originate from bacterial contamination (Zellus, 1999), and (ii) unsaturated fatty acids are usually

esterified to the *sn*-3 position of triacylglycerols in dairy fats, where steric hindrance is limited which enables rapid hydrolysis (Gunstone *et al.*, 1996).

Monitoring of the stable isotope ratios of individual fatty acids during the experimental decay of dairy fats did not reveal any changes in the $\delta^{13}C$ values of individual fatty acids in degraded fats. These observations are important as they support the use of stable carbon isotope ratios of individual fatty acids to determine the identification of degraded archaeological residues.

It was only possible to monitor the incorporation of bacterial fatty acids in the degraded residues during the experimental decay of olive oil as this was the only substrate which did not contain any originally. Branched fatty acids, which are known bacterial markers (Marty *et al.*, 1996), were detected in minor quantities in the degraded olive oil residues, and it is possible that the presence of other bacterial compounds in the degraded residues was responsible for (i) the slight alteration of the stable carbon isotope ratios of fatty acids during the decay of dairy fats, and (ii) the alteration of the unsaturated isomers distribution during the experimental decay of dairy fats.

7.3.2.2 Oxidation products

Oxidation products were only recovered during the experimental decay of olive oil under "hydrolytic" conditions. The mechanism of radical oxidation of oleic acid, the most abundant unsaturated fatty acid in olive oil, is shown in Figure 7.1., and it is evident from this mechanism that the structure of the hydroxy fatty acids that were identified in the degraded olive oil residues can be used as a tool towards the interpretation of degraded residues, as these reflect the original structure of the unsaturated fatty acid they were produced from. This observation is also applicable to 9,10-dihydroxy octadecanoic acid, even if this compound might be produced by a different process. However, as the degradation of unsaturated lipids usually involves "scrambling" of the double-bond position and configuration, the structure of the degradation products might not always exactly reflect that of the original unsaturated compound (Gunstone *et al.*, 1986) hence interpretations should proceed with caution.

7.4 Conclusions

Ester hydrolysis was the main process affecting lipid residues absorbed in potsherds incubated at low to moderate temperatures in a moist burial environment, or buried under field conditions, independently of the nature of the substrate. During burial under "hydrolytic" conditions, the chemical and isotopic composition of the residues was altered as the most labile components were preferentially degraded (unsaturated and low-molecular weight components). "Hydrolytic" conditions will prevail in the majority of burial contexts at archaeological sites in the UK and temperate latitudes. Thus the effects of "hydrolytic" decay process must be considered when interpreting the results of organic residue analyses of lipids in artefacts from such regions.

CHAPTER 8: DISCUSSION: DEGRADATION OF LIPID RESIDUES UNDER "OXIDATIVE" CONDITIONS

8.1 Identification of the processes affecting lipid residues under "oxidative" conditions

8.1.1 Unsaturated lipid oxidation

The absence or much reduced abundance of unsaturated lipids in archaeological residues is thought to be caused by their higher susceptibility to oxidation compared to their saturated counterparts (Evershed *et al.*, 1992). The presence of oxygenated compounds, such as α,ω-dicarboxylic acids, hydroxy acids and polar triacylglycerols was observed during the experimental decay of olive oil under dry and warm conditions (Section 4.4.1), but also during vessel storage (Section 4.5.2). Dairy fats and cod liver oil were also susceptible to unsaturated lipid oxidation during vessel storage (Sections 5.3.1.2 and 6.2.4). The oxidation products observed herein were similar to those observed in archaeological residues recovered fro m arid sites (Regert *et al.*, 1998; Bland, 1999) and have also been reported in ceramics from waterlogged lake sediments.

The range of compounds observed during laboratory decay of olive oil were consistent with their formation *via* radical oxidation of $C_{18:1}$, the main unsaturated fatty acid in olive oil (Gunstone *et al.*, 1986). The first step of $C_{18:1}$ oxidation (Figure 8.1) is hydrogen abstraction from an allylic position to form a radical which can then react with gaseous O_2 to form a range of unsaturated hydroperoxides, whose double-bond position ranges from Δ^8 to Δ^{10}, with the hydroperoxide group on an allylic carbon (Frankel, 1998 and references therein). Hydroperoxide decomposition and intramolecular rearrangement result in the formation of a series of hydroxyacids, but also ketoacids and epoxyacids, which can then undergo oxidative cleavage to form two series of α,ω-dicarboxylic acids and of short-chain fatty acids, both dominated by the C_9 compounds. The presence of different isomeric hydroxy acids, or different α,ω-dicarboxylic acids indicates that chemical oxidation took place, whereas biological oxidation would have yielded specific compounds (German *et al.*, 1992). Experimental decay of olive oil produced all the compounds sited above in significant quantities. Oxidative decay of cod liver oil yielded a series of α,ω-dicarboxylic acids and dihydroxy acids (Section 6.2.4), and oxidative decay of dairy fats yielded a series of α,ω-dicarboxylic acids (Section 5.3.1.2), which

illustrates that the oxidative processes observed with olive oil as a substrate also affect other commodities, such as cod liver oil and dairy fats.

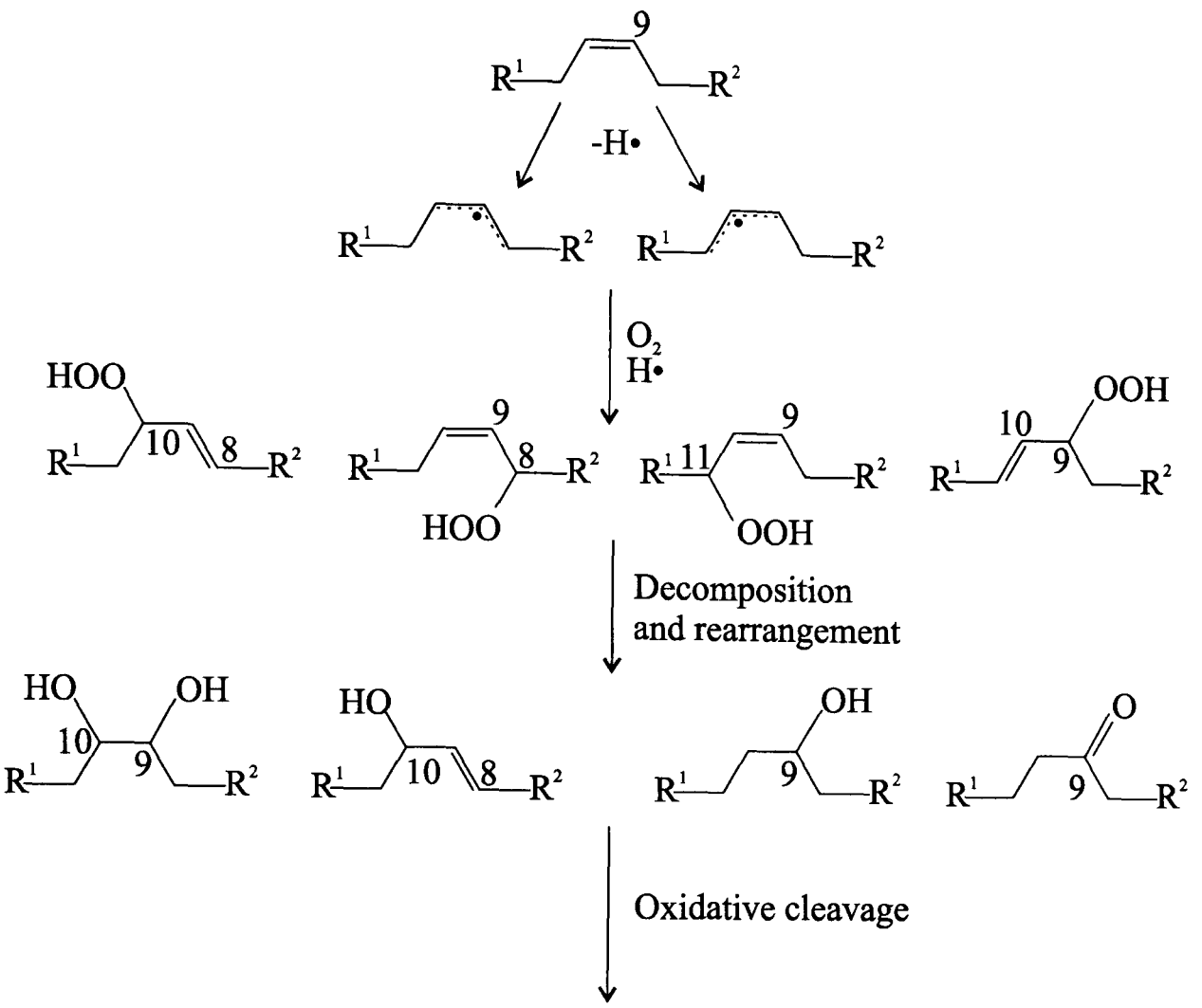

Figure 8.1: Mechanism of oleic acid radical oxidation during experimental decay under "oxidative" conditions. $R^1 = C_7H_{15}$, $R^2 = C_7H_{13}O_2$.

There was no significant difference in the rate and extent of decay, or in the nature of the degradation products, between the experimental decay of olive oil under aerobic or anaerobic conditions (Section 4.2.1). This was unexpected because of the importance of oxygen in the mechanism of unsaturated lipid radical oxidation. However, Crapiste and coworkers (1999) studied the oxidation of sunflower oil during storage in open and capped flasks, and demonstrated that the most important factor governing the rate and extent of oxidation was the surface area of the oil exposed to air. Ceramics are very porous and have a very high surface area, and it is very likely that the physical state of the absorbed residues is reminiscent of a thin film, with a very high surface area, thus enducing high oxidation rates.

Residues extracted from potsherds incubated in a clean flask containing no mushroom compost (Section 4.5.2) were affected by "oxidative" decay of the unsaturated components. The presence of oxidation products has been reported in residues extracted from ethnographic vessels used for the storage and cooking of pork fat and olive oil (Dudd, 1999) and in residues extracted from unglazed ceramic lamps (Bland, 1999) which have never been buried, and therefore, have never been in contact with soil microorganisms. As the conditions described herein are very unfavourable to bacterial activity, it is extremely likely that the "oxidative" processes seen were essentially chemical mediated.

8.1.2 Ester hydrolysis

As the formation of free fatty acids was observed during decay under "oxidative" conditions, it is likely that ester hydrolysis took place to a limited extent under these conditions. This is consistent with observations made by Aubourg (1999) in the case of fish lipids. Interaction of clay minerals on water and triacylglycerols absorbed within the clay matrix is also thought to result by the hydrolysis of the triacylglycerol (Theng, 1974; Bland, 1999).

8.2 Preservation of the chemical and isotopic composition of the absorbed residues

8.2.1 Triacylglycerols

The triacylglycerol distribution of the residues was altered in two ways: (i) there was a preferential loss of the more unsaturated compounds (e.g. Section 5.3.1.2), and (ii) production of polar compounds resulted in loss of chromatographic resolution due to broadening and overlapping of the peaks (e.g. Sections 4.4.1.1 and 5.3.1.2).

Depletion of the triacylglycerols as experiments progress was generally very rapid, which is consistent with the scarcity of triacylglycerols in archaeological residues from arid sites (Bland, 1999). Furthermore, the more unsaturated triacylglycerols were preferentially depleted during the experimental decay of dairy fats under "oxidative" decay, resulting in the significant alteration of the triacylglycerol distributions. This is consistent with observations made by Dudd (1999) who compared the triacylglycerol

distribution of archaeological animal fats with the more saturated fraction of fresh animal fats, and concluded that decay resulted in the preferential depletion of the more unsaturated triacylglycerols.

The production of polar triacylglycerols is usually the first step of any oxidative processes affecting oils and fats (Frankel, 1985; Hopia, 1993a and b; Lampi and Pironen, 1999; Márquez-Ruiz *et al.*, 1998) and is often followed by the formation of polymers (Muizebelt and Nielen, 1996; Márquez-Ruiz *et al.*, 1998; Martin *et al.*, 1998; Artz *et al.*, 1997), as with drying oils (Mallégol *et al.*, 1999; Mills and White, 1994). Oxidation of $C_{18:1}$ clearly occurred while the unsaturated fatty acid was still esterified to the glycerol backbone, yielding a highly complex mixture of relatively polar triacylglycerols unresolvable by HTGC. Low molecular weight UCMs have been observed in a number of vessels from Mere Lake Village, Somerset, UK and Fuller's Hill, Great Yarmouth, UK, both settlements where fish is thought to have been an important component of the diet (Dudd, 1999). It is thought that the oxidation of fish oil, containing mainly long-chain polyunsaturated acyl lipids, would yield a complex mixture of polar compounds. This hypothesis was supported by the $\delta^{13}C$ values of individual fatty acids forming part of the UCM which compared favourably with values obtained for fresh fish oil (Copley, Dudd and Evershed, unpublished results). No complex mixture was observed during the experimental decay of cod liver oil, but the experiment was only allowed to run for a limited period of time, such that the residues were not extensively degraded.

8.2.2 Degradation products

8.2.2.1 Free fatty acids

All residues incubated under "oxidative" conditions showed a reduced abundance of the unsaturated components, especially the unsaturated fatty acids, but the relative abundance of the saturated fatty acids was not altered significantly (e.g. Section 4.4.1.2). The preferential depletion of unsaturated fatty acids has been reported in ceramics (Regert *et al.*, 1998a and 1998b, Bland, 1999) and mummies (Gülaçar *et al.*, 1989 and 1990; Buckley and Evershed, 1999) excavated mostly from arid sites. Furthermore, degraded cow butter residues incubated under "oxidative" conditions showed preferential depletion of the *cis*-isomers and a corresponding increase in the relative abundance of the more stable *trans*-isomers (Coultate, 1996; Section 5.3.1.3). Alteration of the double-

bond position and configuration in unsaturated fatty acids has been reported to accompany oxidative degradation (Gunstone *et al.*, 1986; Mallégol *et al.*, 1999) *via* the formation of radical species, and is probably catalysed by the ceramic minerals (Stefanova and Disnar, 2000). Bacterial reworking of the unsaturated fatty acids is very unlikely to have occurred because the incubation conditions described herein are very unfavourable to the maintenance of bacterial activity (Parshotam *et al.*, 2000; Bottner *et al.*, 2000; Wandsnider, 1997). This is also evidenced by the absence of bacterial markers from degraded olive oil residues that were incubated under "oxidative" conditions (Section 4.4.1.2).

8.2.2.2 Oxidation products

As interpretation of the origin of lipid residue in archaeological pottery has traditionally been achieved by investigating the relative abundance of fatty acids in solvent extracts after saponification, oxidation products have seldom been characterised. Furthermore, oxidation products are thought to be more water-soluble than their intact homologues due to their higher polarity, and are therefore thought to be easily lost from artefacts by groundwater leaching (Regert *et al.*, 1998a). So far, oxidation products have mainly been reported in potsherds (Regert *et al.*, 1998; Bland, 1999) and mummies (Gülaçar *et al.*, 1989 and 1990; Buckley and Evershed., 1999) mostly recovered from arid locations and include α,ω-dicarboxylic acids, hydroxy acids and ketoacids.

Alkaline hydrolysis of extracted potsherds yielded compounds not always detectable in the extractable fraction, and, thus, provided further information regarding the commodities processed in the vessel, as illustrated by the numerous ceramic lamps presented by Bland (1999). Previous investigators have demonstrated that "bonding" of oxidation products to the ceramic matrix protected them from loss by solubilisation in percolating water, and thus, enhanced their preservation (Regert *et al.*, 1998).

Study of oxidation products recovered both from the extractable and "bound" fractions yielded information regarding the presence and the structure of unsaturated acyl lipids initially present in the residues. For instance, the position of the hydroxy functions in vicinal dihydroxyacids reflected the original position of the double bond, whereas their stereochemistry reflects the double-bond original configuration (Section 6.2.4). However, most radical reactions, such as oxidation, are often accompanied by double-bond

movement and/or stereomutation (Martin *et al.*, 1998), so that the structure of the degradation products might not always exactly reflect that of the original unsaturated compound. α,ω-dicarboxylic acids have been previously recovered in similar proportions from the ceramics from arid sites (Regert *et al.*, 1998; Bland, 1999). They are formed by oxidation of unsaturated fatty acids, by: (i) direct cleavage of the double-bond, (ii) hydration of the double-bond followed by cleavage, (iii) ω-oxidation followed by double-bond cleavage or (iv) ω-oxidation, hydration of the double-bond followed by cleavage (Gillan and Johns, 1982). α,ω-Dicarboxylic acids can also be formed from polyunsaturated triacylglycerols (Passi *et al.*, 1993). The α,ω-dicarboxylic acids and short-chain fatty acids distributions were dominated by the C_9 compounds, suggesting that they were formed from a fatty acid with an unsaturation at position 9, such as oleic acid which is the main unsaturated fatty acid present in olive oil or dairy fats (Sections 4.4.1 and 5.3.1.2).

In the ceramic matrix, the first step of oxidative decay appeared to be the oxidative degradation of the unsaturated triacylglycerols, followed by "bonding" of the oxidised species into the matrix, and, finally, the subsequent release of the α,ω-dicarboxylic acids. The "bound" fraction is however not protected from oxidation as its composition changes with time to include a lower proportion of intact fatty acids and predominantly oxidised species (Section 4.4.2).

8.3 Conclusions

The recovery of oxidation products from both the solvent-extractable and "bound" fractions obtained after incubation of different substrates, accompanied by the preferential depletion of the unsaturated components of the residues, indicates that unsaturated lipid oxidation was the main process affecting absorbed residues under "oxidative" conditions. The distribution of the oxidation products identified in the degraded residues indicates that oxidation was chemically driven, above all as "oxidative" conditions were not favourable to bacterial activity. The chemical composition of the residues was significantly altered by "oxidative" processes that resulted in the preferential depletion of the more unsaturated components of the residues. The structure and distribution of the oxidation products recovered in either the solvent-extractable or "bound" fractions, however, reflected the original structure of the unsaturated fatty acid they had been produced from, and it is very likely that the presence

of oxidation products associated with archaeological ceramics could be used towards the identification of degraded residues.

CHAPTER 9: OVERVIEW AND RECOMMENDATIONS FOR FUTURE WORK

9.1 Overview

Gas chromatographic and mass spectrometric analyses of degraded residues extracted from archaeological and experimental ceramics have been carried out to investigate degradative processes affecting absorbed residues. The main body of the thesis was concerned with the experimental decay of 3 commodities representative of food groups that would have been commonplace in Antiquity, namely olive oil, dairy fats and cod liver oil, under laboratory and field conditions, in order to determine the pathways and products of chemical and enzymatic lipid degradation in unglazed potsherds. The overall aim of the thesis was to rationalise how diagenetic processes affect the composition of absorbed lipid residues in different sites and contexts within sites, and to compare the results of the laboratory and field experiments to archaeological residues in order to validate the interpretation of the latter.

9.1.1 Physical alterations of lipid residues during vessel use and/or burial

Physical processes affecting lipids absorbed in unglazed ceramics, e.g. absorption of soil lipids or loss of some components through groundwater leaching, can potentially alter the composition of archaeological residues and prevent their correct identification. Experimental work carried herein has demonstrated that unglazed ceramics were unlikely to absorb lipid components from their burial environment in significant quantities. This is consistent with previous findings (Heron and Evershed, 1991) and confirms that the majority of lipid components absorbed in archaeological ceramics derive from the processing of animal or vegetable commodities in the vessels.

9.1.2 Experimental decay of olive oil absorbed in unglazed ceramics under laboratory conditions

Olive oil was an important commodity in antiquity (Meeks, 1993) and was traded extensively in ceramic vessels (Sealey and Tyers, 1989) but has seldom been identified in the archaeological record. The experimental decay of olive oil absorbed in unglazed ceramics has been carried out under different laboratory conditions chosen to promote a

range of chemical and biological decay mechanisms. Two categories of experimental conditions were identified, depending on the degradation processes they promoted.

(i) "Hydrolytic" conditions

Cold and wet incubation conditions promoted ester hydrolysis of the triacylglycerols. Degraded residues recovered during experiments conducted under "hydrolytic" conditions mainly contained tri-, di- and monoacylglycerols and free fatty acids, thus resembling the majority of residues recovered from temperate regions (e.g. Charters, 1996; Dudd, 1999). The free fatty acid and triacylglycerol distributions were mostly preserved under these conditions, but the preferential loss of the unsaturated fatty acids was observed in the majority of the experiments, especially in those conducted at higher temperature. Several hydroxy fatty acids resulting from the oxidation of the most abundant unsaturated fatty acid in olive oil, oleic acid, were identified in degraded residues incubated at high temperature.

(ii) "Oxidative" conditions

Warm and dry conditions favoured unsaturated lipid oxidation of the residues resulting in the dramatic alteration of their compositions, through the preferential loss of the more unsaturated species. The relative abundance of triacylglycerols decreased very rapidly during experiments conducted under "oxidative" conditions, and degraded residues incubated under these conditions did not contain any triacylglycerols. This is consistent with observations made by Bland (1999) on the composition of degraded residues recovered from an extremely arid site. A range of oxidation products, including α,ω-dicarboxylic acids, short-chain fatty acids, several hydroxy acids and polar triacylglycerols, were identified in both the solvent-extractable and "bound" fractions recovered during these experiments. Overall, the composition of the residues closely resembled that of residues recovered from arid sites (Bland, 1999).

9.1.3 Experimental decay of dairy fats absorbed in unglazed ceramics under laboratory and field conditions

Dairy fats are characterised by the presence of low-molecular weight triacylglycerols containing as few as 26 acyl carbon atoms that release short-chain fatty acids upon

hydrolysis. These characteristic low molecular weight components are highly labile, and it has been shown that they become very reduced in abundance in degraded dairy fats (Dudd *et al.*, 1998; Dudd and Evershed, 1998; Dudd, 1999). Other properties of dairy fats can be considered for the identification of degraded dairy fats, such as $\delta^{13}C$ values of individual fatty acids, and double-bond position and configuration in unsaturated fatty acids (Dudd, 1999). Dairy fats were submitted to experimental decay under laboratory and field conditions in order to test the robustness of these properties during vessel/sherd burial. The conditions for the laboratory experiments were chosen to promote ester hydrolysis and unsaturated lipid oxidation, and were similar to that under which olive oil residues were incubated.

(i) "Hydrolytic" conditions

As expected, triacylglycerol hydrolysis was the dominant degradation mechanism affecting dairy fats incubated under "hydrolytic" conditions, or buried under field conditions. Experiments conducted under "hydrolytic" and field conditions showed a decrease in the relative abundance of the low-molecular weight triacylglycerols (containing less than 46 acyl carbon atoms). This process is very likely to affect archaeological samples buried under cold and wet conditions, and would result in triacylglycerol distributions lacking the low-molecular weight components, such as those observed in the ceramics from Eton and Crick (Figure 9.1), but also in several pottery assemblages reported elsewhere (Dudd and Evershed, 1998; Dudd, 1999).

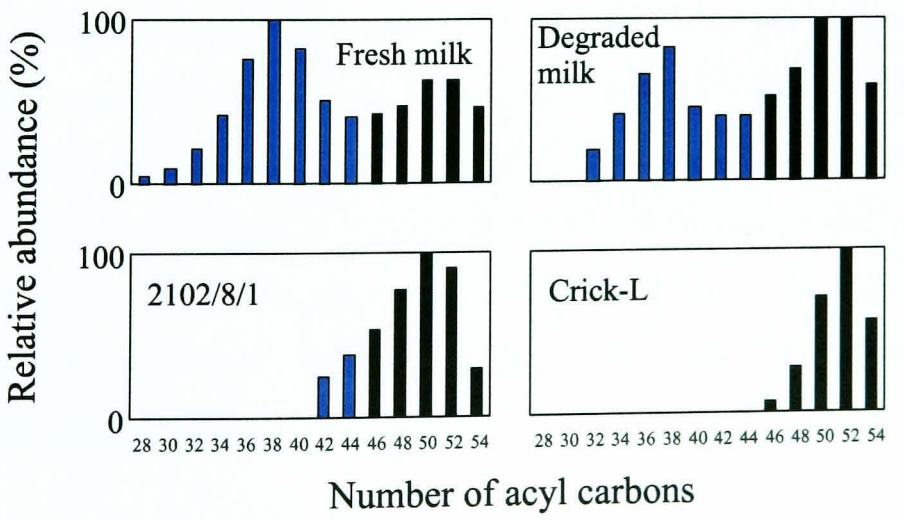

Figure 9.1: Triacylglycerol distributions in fresh milk, degraded milk buried for 15 days, and in the total lipid extracts from the potsherds 2108/1 and Crick-L.

The free fatty acid distribution of the degraded dairy fats were also different to the distributions that would have been obtained from saponification of the intact dairy fats as: (i) they lacked the short-chain components characteristic of dairy fats, and (ii) the unsaturated $C_{18:1}$ was preferentially released from the triacylglycerols. Here again, the free fatty acid distributions observed in the degraded dairy residues resemble distributions reported in several pottery assemblages (Dudd, 1999, and Chapter 3).

Degraded dairy residues incubated under "hydrolytic" conditions or buried under field conditions exhibited $\delta^{13}C$ values for $C_{16:0}$ and $C_{18:0}$ that were comparable to the values for these fatty acid in the intact dairy fats used as substrate (Sections 5.2.1.3, 5.3.1.4 and 5.3.2.4). These observations support the use of $\delta^{13}C$ values of individual fatty acids for the identification of degraded archaeological residues.

(ii) "Oxidative" conditions

Degraded dairy residues incubated under "oxidative" conditions became very depleted in the more unsaturated triacylglycerols and fatty acids, with the unsaturated fatty acids with double-bonds in the *cis* configuration more affected than those with the double-bonds in the *trans* configuration (Figure 9.3). The preferential depletion of the *cis* configured fatty acids could lead to distribution of $C_{18:1}$ isomers similar to those observed in Eton and Crick, and in other pottery assemblages (Dudd, 1999). The double-bond configuration and position in unsaturated fatty acids is being used increasingly to distinguish between ruminant and non-ruminant fats (Evershed *et al.*, 1997; Mottram *et al.*, 1999; Dudd, 1999), but does not appear to be an entirely robust criteria during decay. Nevertheless, some samples from Crick and Eton exhibited very good preservation of the $C_{18:1}$ isomers distribution (Chapter 3). Furthermore, the reduction in abundance of the $C_{18:1}$ isomers in the *cis* configuration resulted in an increase of the relative abundance of those in the *trans* configuration which are characteristic of dairy fats (Figure 9.2). Overall, the distribution of $C_{18:1}$ isomers remain a useful tool for the identification of degraded archaeological residues.

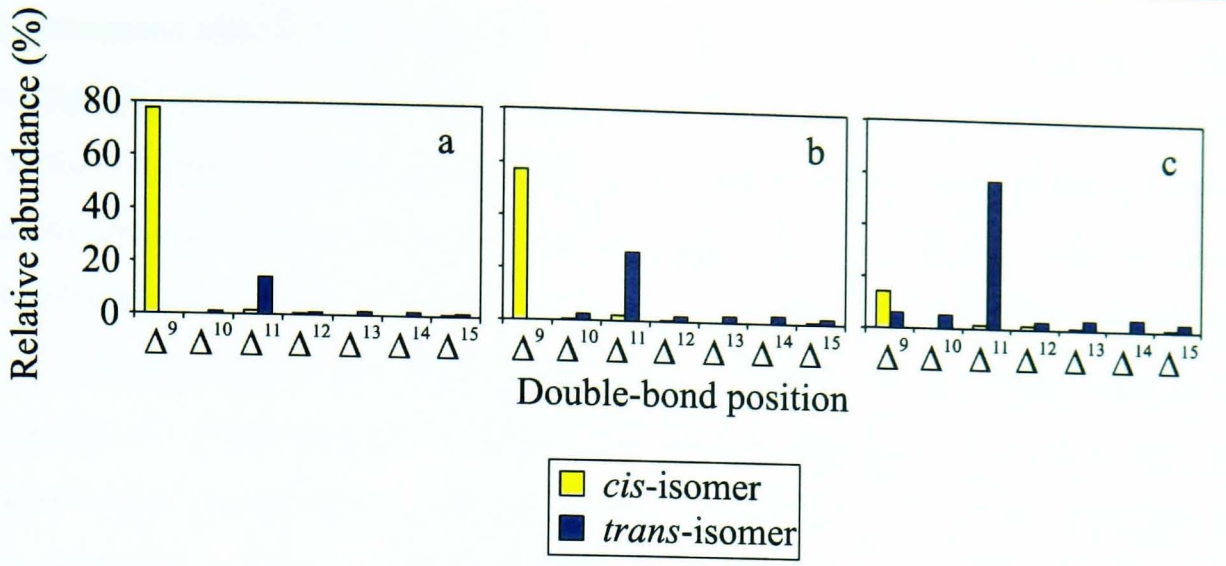

Figure 9.2: Distribution of the $C_{18:1}$ isomers in a) fresh cow butter, b) cow butter incubated under "oxidative" conditions for 349 days, and c) Crick-B.

Similar to what was observed during the experimental decay of olive oil under "oxidative" conditions, oxidation products including short-chain fatty acids and α,ω-dicarboxylic acids were identified, and the presence of polar triacylglycerols was also apparent, resulting in the loss of chromatographic separation between triacylglycerol peaks.

9.1.4 Experimental decay of cod liver oil absorbed in unglazed ceramics under laboratory conditions

Cod liver oil was chosen as a substrate because of its reported high degree of unsaturation. Incubation conditions for this substrate was chosen so as to promote unsaturated lipid oxidation and ester hydrolysis, and were similar to those under which olive oil residues have been incubated. The unsaturated components of cod liver oil were significantly depleted in all degraded residues, illustrating the importance of oxidative processes in the decay of this commodity. However, very few oxidation products were detected in either the solvent-extractable or "bound" fractions recovered after incubation. The most likely explanation is that the main products of oxidation were volatile species that were not recovered by solvent-extraction. One cod liver oil residue nevertheless showed a range of α,ω-dicarboxylic acid and a series of dihydroxy fatty acids whose structure exactly matched the structure of the unsaturated fatty acids originally present in the cod liver oil. The presence of vicinal dihydroxy fatty acids in pottery extracts has been exploited by Bland (1999) to determine the likely structure of the unsaturated fatty acids they have been produced from, and thereby identified a range of vegetable oils used as fuel in ceramic lamps.

Overall, processes affecting absorbed lipid residues include removal or attack of the more labile components, i.e. low molecular weight and unsaturated (especially with double-bonds in the *cis* configuration) components, and are mostly accompanied by an overall decrease in the total lipid content of potsherds. Recovery of oxidation products in the solvent-extractable or "bound" fractions can provide information regarding the original presence of unsaturated components in the residues. Most of the criteria used in the identification of archaeological residues, especially fatty acid and triacylglycerol distribution were significantly affected by decay. The extent of this alteration was strongly dependant on the incubation or burial conditions, which have to be taken into consideration when interpreting the results of analyses of archaeological residues.

9.2 Recommendations for future work

9.2.1 Extension of the range of oils, fats, and pure compounds to be experimentally degraded

Experimental work presented herein demonstrated that the same decay processes affect different commodities. However, extension of the present work to a wider range of commodities would enable generalization of this conclusion. For example, another worker in the Bristol laboratory is currently investigating the decay of beeswax under laboratory conditions. The experimental decay of pure compounds in conditions similar to those described herein would enable to simplify interpretations by limiting the range of degradation products, thereby allowing the degradation pathways to be established more precisely, and would aid the identification of complex species, such as the polar triacylglycerols produced by oxidation of olive oil.

9.2.2 Extension of the range of incubation conditions

As a further aid to the interpretation of the results of archaeological residues, extension of the range of the conditions of incubation would be of value, to include factors that are known to govern the rate and extent of decay of soil organic matter, e.g. soil pH (Curtin *et al.*, 1998), soil organic matter (Alexander, 1999), aeration level (DeLaune *et al.*, 1991). With this in mind, the experimental decay of dairy fats under field conditions, in soils of different pH and geological bed rock has been initiated.

Incubation of potsherds containing C_3 lipids in soils on which C_4 vegetation had been grown would allow contamination from soil or bacterial lipids to be readily recognised since these compounds would be enriched in their $\delta^{13}C$ values compared to the original lipid residue.

9.2.3 Characterisation of the components of the unresolved complex mixture

HPLC and/or LC-MS have been successfully applied to the analysis of triacylglycerols (Mottram, 1999, and references therein), and is likely to be useful for the determination of the molecular weight and structure of the polar triacylglycerols forming part of the unresolved complex mixture observed during the experimental decay of olive oil (Wolff *et al.*, 1991; Neff and Byrdwell, 1998; Byrdwell and Neff, 1999). This approach might be especially valuable in degradation experiments using single compounds.

9.2.4 $\delta^{13}C$ values of individual fatty acids in degraded residues

$\delta^{13}C$ values of individual fatty acids are being increasingly used in the identification of archaeological residues (Evershed *et al.*, 1994 and 1997b; Dudd, 1999). The experimental work presented herein showed that there were no significant alterations of the $\delta^{13}C$ values of individual fatty acids in degraded residues, but would benefit from an extension of the range of commodities monitored and of the time-scale of the experiments.

9.2.5 Phospholipids in absorbed residues

Phospholipids are present in most animal and vegetable commodities, but are not detectable using HTGC (Gunstone *et al.*, 1986). It would be interesting to conduct a series of experiments investigating: (i) whether phospholipids can be absorbed in the matrix of unglazed ceramics during vessel use, and (ii), if, during vessel burial, they yield degradation products, especially free fatty acids and oxidation products, which contribute to the total lipid extract. This could be investigated by submitting blank potsherds dosed with pure phospholipid standards to experimental decay. Saponification of the total lipid extract recovered from these potsherds would release the fatty acids from the phospholipids backbone, and would allow their analysis by GC, together with other degradation products formed during decay.

9.2.6 Nature of the binding between the "bound" fraction and the ceramic matrix

Further work should include alkaline hydrolysis of some extracted potsherds using $Na^{18}OH/H_2{}^{18}O$ to investigate if the components of the "bound" fraction are linked to the ceramic matrix via ester bonds, as their mass spectra would show the extent of incorporation of the heavy oxygen isotope.

9.2.7 Other ceramics

Metals cations present in the ceramic matrix are thought to play an important role regarding the catalysis of chemical and biological decay reactions (Davídek *et al.*, 1990). It would then be interesting to conduct experimental decay of one commodity in several ceramics having different metal contents in order to investigate the influence of these metals on the rate and extent of decay of absorbed lipids.

9.2.8 Chemical and isotopic composition of dairy products

Milk can be transformed into a variety of products, including yoghurt and cheese which are produced by bacterial action (Ainsworth, 1996). Detailed analyses of dairy products prepared in the laboratory would allow any changes in the chemical or isotopic composition of the lipid components of these products to be determined, or if any characteristic new components are produced during preparation. This knowledge could then be applied to the identification of different dairy products in archaeological ceramics.

9.2.9 Novel marker for degraded marine oils

Two characteristic isoprenoid compounds were identified in the degraded cod liver oil residues, namely 4,8,12-trimethyltridecanoic acid and 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid), shown in Figure 9.3. These compounds have been reported in several other fish oils (e.g. dogfish in Kang *et al.*, 1998) and it is likely that they could be used as novel markers for degraded marine oils in archaeological ceramics. Their presence in pottery assemblages excavated from coastal sites. is currently investigated by other workers from the Bristol laboratory.

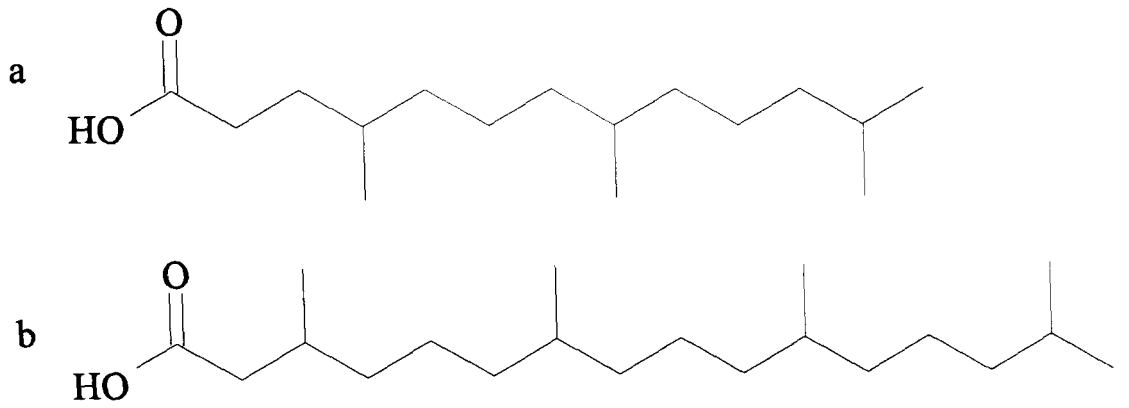

Figure 9.3: Chemical structure of a) 4,8,12-trimethyltridecanoic acid and b) 3,7,11,15-tetramethylhexadecanoic.

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